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ORIGINAL ARTICLES

INHIBITION OF EXPERIMENTAL SKIN WHEAL BY SOME ATARACTIC AND ANTIHISTAMINIC DRUGS

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A PREVIOUS PUBLICATION[1] reports the experimental demonstration of the capacity of chlorpromazine to partially suppress the wheal induced in the human skin by histamine injection. In the following report, a group of three "ataractic" and one "antihistaminic" drugs are compared in this respect.

The original basis for these studies lay in the observation by the senior author of clinical improvement in selected cases of chronic urticaria following the exhibition of chlorpromazine (Thorazine, Largactil). Such a wide range was observed, however, in the degree of clinical response to the drug exhibited by different patients that it appeared unlikely that the therapeutic benefit could be confidently ascribed to any direct pharmacological action of chlorpromazine upon skin whealing; even though the latter action is experimentally demonstrable.[1]

It is notable that one other "tranquillizer," hydroxyzine (Atarax), an ataractic whose chemical structure differs from chlorpromazine, has recently been reported to be very effective in the treatment of some cases of chronic urticaria[2, 3] and to possess antihistaminic properties.[4] Therefore the following drugs were chosen for comparison with chlorpromazine in respect to their wheal-suppressive activity: (1) perphenazine (Trilafon)—an ataractic and an analogue of chlorpromazine derived from the same phenothiazine base; (2)

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hydroxyzine (Atarax)—an ataractic structurally quite different from chlorpromazine and perphenazine, derived from a diphenyl methane base; (3) chlorphenamine maleate (Chlor-Tripolon, Chlor-Trimeton)—an antihistamine widely accepted as satisfactorily clinically effective.

GENERAL METHOD

Histamine was used as the whealing agent. A quantitative measure of response, the mean skin wheal diameter, was used rather than an end-point titration. This method has elsewhere been described in more detail.[1] Dose-response regression lines for graduated doses of histamine were derived from the wheal diameter data. Changes in the positioning of these lines following

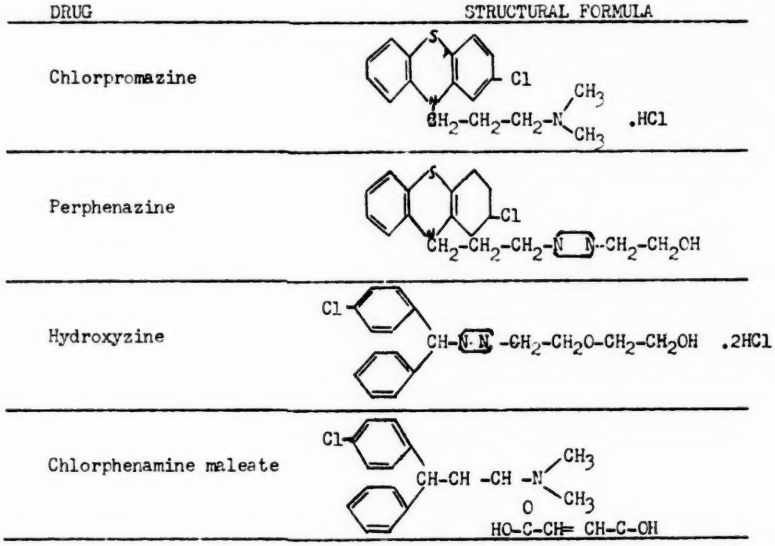

Fig. 1.

pretreatment of the experimental subjects with each of the above-mentioned ataractic and antihistaminic drugs were investigated. Antiwhealing factors tend to displace the line downward. Wheal-enhancing factors tend to displace it upward. The magnitude of displacement provides a basis for measurement of relative potency.

Statistical analysis was performed by an analysis of paired data.

EXPERIMENT

Four young men were used as test subjects. Six separate testing occasions were planned with a five-day interval between test occasions: an untreated control at the onset and after completion of the series, and four treated states. Each test subject received each of the four drugs under study. The individual drug doses were selected as being the maximum dose recommended by the manufacturers for usual clinical use for a period of twenty-four hours in nonpsychotic patients. A comparison in this way was considered to be of more value in a direct clinical manner than a milligram-for-milligram comparison.

Each individual took his oral medication over the twenty-four hours preceding the testing day, the last dose being ingested two hours before the skin test procedure. Drug dosage used is summarized in Table I. Histamine dilutions were made from Abbott's histamine diphosphate solution one hour before each testing occasion. Three testing doses of histamine: 1 microgram, 0.5 microgram, and 0.25 microgram of the base, each contained in 0.05 ml. of solution, were injected intradermally into each test subject's back in the manner illustrated in Fig. 2. This design eliminates bias due to the effect on wheal size of body site of the test[4, 5] by permitting each test strength of histamine to occur the same number of times in each row and each column.

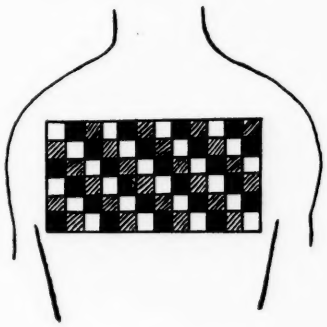

Fig. 2.—Skin test pattern.

TABLE I

DRUG	RECOMMENDED DOSAGE RANGE (MG.)	DOSE USED (MG.)
Hydroxyzine (Atarax)	30-75 10-25 every 8 hr.	75 25 every 8 hr.
Chlorpromazine (Thorazine, Largactil)	75-150 25 every 8 hr.—every 4 hr.	150 50 every 8 hr.
Chlorphenamine maleate (Chlor-Tripolon, Chlor-Trimeton)	8-24	24 8 every 8 hr. (repetabs)
Perphenazine (Trilafon)	12-24 4-8 t.i.d.	24 8 every 8 hr.

Drug doses given every 8 hr. over the twenty-four hours preceding the test. Last dose given two hours preceding the test.

The control (untreated state) observations at the onset and completion of the experiment were considered advisable to permit evaluation of any possible skin "desensitization" to the histamine. The data from both controls are listed with the results, but due to a technical error in the reading of the wheals of Control 1, they are not considered comparable with the rest of the data. The comparisons in Tables II and III, and Fig. 3, are therefore between each of the

treated states and Control 2. Fortunately, the data indicate that, if any exhaustion of the reacting mechanism after repeated skin testing did in fact occur, it was not sufficient to obscure the drug effects under observation.

OBSERVATIONS

The results for all four subjects were average and are outlined in Table II. Dose-response lines for these mean data are plotted on a logarithmic scale in Fig. 3. Satisfactory linear regression of response on log-dose histamine is evident throughout the experiment except after treatment with hydroxyzine. This drug appeared to totally suppress the whealing response. The small wheal observed is believed to be factitious.

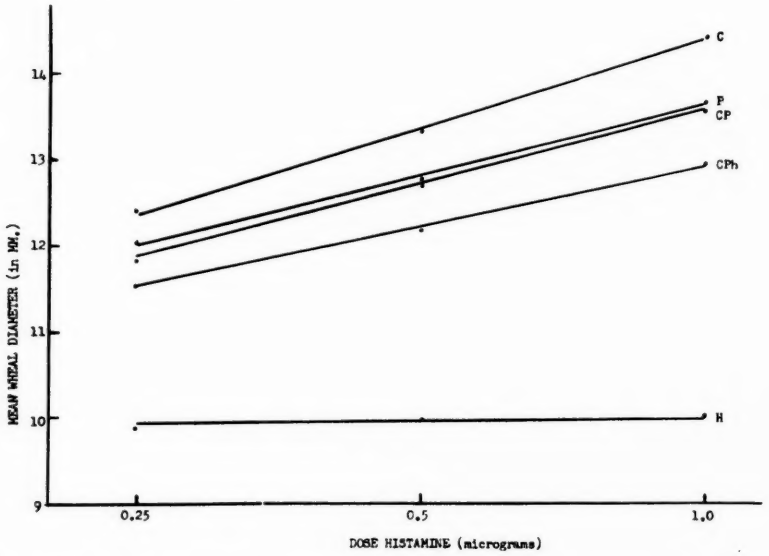

Fig. 3.—Log-dose histamine versus mean wheal diameter for treated and untreated states. (Average of four subjects.)

C—Control (untreated). *P*—Perphenazine. *CP*—Chlorpromazine. *CPh*—Chlorphenamine. *H*—Hydroxyzine.

TABLE II. HISTAMINE IN HUMAN BEINGS

DRUG	DOSAGE (MG.)	MEAN WHEAL DIAMETER (AVERAGES OF 4 SUBJECTS) HISTAMINE 1 μG	HISTAMINE 0.5 μG	HISTAMINE 0.25 μG
Control 1	0	13.08 mm.	12.23 mm.	11.53 mm.
Hydroxyzine	75	9.99	9.99	9.95
Chlorphenamine	24	12.90	12.20	11.58
Chlorpromazine	150	13.53	12.72	11.88
Perphenazine	24	13.63	12.71	12.08
Control 2	0	14.36	13.33	12.41

The data obtained with the strongest test dose of histamine (1 microgram) were statistically analyzed for each test individual separately and are given in Table III. The difference between each of the treated states and the untreated control proved to be highly significant. The probability that these differences are due to chance is less than 1 per cent.

An expression of relative potencies for the drugs tested may be calculated by the method described in Emmens' text.[6] This, as illustrated in Fig. 4, gives an indirect indication of relative potencies, i.e., in terms of the decreased activity of the histamine testing solution after pretreatment by the inhibitory drugs. These ratios are computed, with 95 per cent confidence limits, as follows:

0.374 (0.317—0.443) following chlorphenamine
0.598 (0.539—0.663) following chlorpromazine
0.663 (0.557—0.718) following perphenazine

That is to say, under these experimental conditions, the dose of chlorphenamine produced a reduction of about two thirds in the histamine test solution's activity; chlorpromazine and perphenazine were somewhat weaker, in that they each reduced activity by about two fifths. Unfortunately hydroxyzine

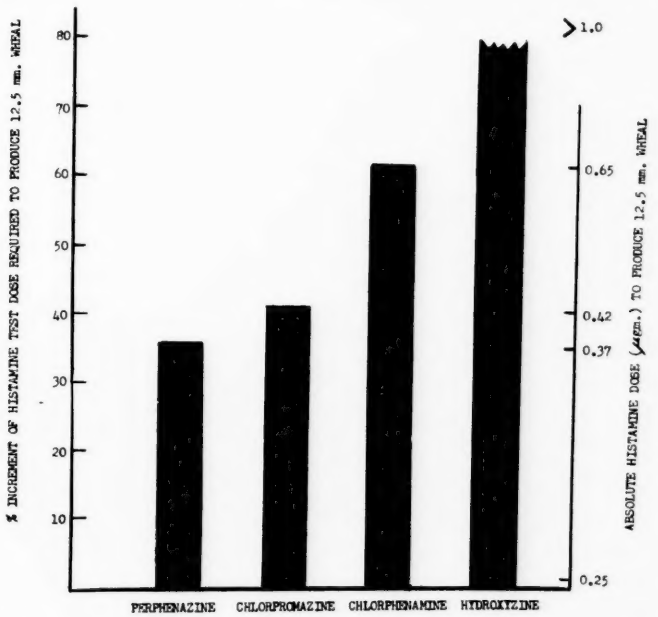

Fig. 4.—Relative antiwhealing potency in terms of the required increment of histamine base to produce an arbitrary standard 12.5 mm. wheal. In the untreated state this size wheal is produced by 0.25 μg histamine base.

TABLE III. HISTAMINE WHEAL IN HUMAN BEINGS—TREATED VS. UNTREATED GROUPS
(TEST STRENGTH—1.0 μG HISTAMINE BASE)

	DAY 1	DAY 3	DAY 8	DAY 13	DAY 18	DAY 23
TEST SUBJECT	CONTROL 1*	HYDROXYZINE (75 MG.)	CHLOR-PHENAMINE (24 MG.)	CHLOR-PROMAZINE (150 MG.)	PERPHENA-ZINE (24 MG.)	CONTROL 2
A	13.32 mm.	9.68 mm.	12.48 mm.	13.22 mm.	13.30 mm.	14.05 mm.
B	13.57	9.76	13.45	13.70	14.23	14.75
C	12.83	10.07	13.14	13.88	14.09	14.96
D	12.62	10.46	12.51	13.32	12.90	13.68
Mean	—	9.99	12.90	13.53	13.63	14.36

Drug doses given every 8 hr. over the twenty-four hours preceding the test. Last dose given two hours preceding the test.

$P = < 0.001$ for all drugs in all four subjects as compared with Control 2.

*Omitted from final calculations.

is so very potent that the histamine regression line was depressed so far as to preclude calculation of a relative potency for hydroxyzine—which would have been interesting.

While it is true that a single, arbitrarily selected dose of each antiwhealing drug has been used in the experiment described here, this dose is the maximum clinically tolerated dose (MCTD) for the ordinary nonpsychotic patient. The potency ratios are therefore with respect to this MCTD and could be increased for any particular one of the drugs by raising its dose relative to the rest. This is impractical as "side effects" limit the use of higher doses.

As long as the drugs are all compared on the basis of their respective MCTDs or some constant fraction of the MCTD, one would expect on a priori grounds that these potency ratios would remain the same—provided of course that this particular technique for comparison were used. Some further studies by our group[1] confirm this a priori assumption by showing parallel regression lines for these drugs over a wide dosage range. This also accords with the experience of Bain and his colleagues.[2]

CONCLUSIONS

In the doses used, the relative effect of the four drugs tested was the same in all four experimental subjects. It is apparent by inspection of Fig. 3 that the antiwhealing activity is most marked with hydroxyzine. Chlorpromazine and perphenazine, the two phenothiazine derivatives, have about the same order of antiwhealing activity. Chlorphenamine maleate exceeds these latter in activity but still falls short of hydroxyzine in this regard.

SUMMARY

A direct inhibitory action on the human skin whealing mechanism is demonstrated for the four ataractic and antihistaminic drugs tested. In the doses used, hydroxyzine greatly exceeds the phenothiazine type ataractics, and the antihistamine, chlorphenamine maleate, in wheal depressant potency.

This work has been made possible by the summer student research fellowship plan of the Allergy Foundation of America. Additional support, herewith gratefully acknowledged, was provided by the Victoria Hospital Allergy Clinic and by the following companies who donated the drugs tested: Schering Corporation, Pfizer Corporation, and Poulenc Freres. We also acknowledge the valued assistance of Dr. Carol Buck of the Department of Psychiatry and Preventive Medicine; Professor J. A. F. Stevenson, Department of Physiology, University of Western Ontario Medical School; and Miss Muriel Dennison, Allergy Laboratory, Victoria Hospital.

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THE MECHANICAL PROPERTIES OF THE LUNGS IN EXPERIMENTAL ASTHMA IN THE GUINEA PIG

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NUMEROUS investigators[1, 2, 3] have demonstrated that a finely sprayed antigen produces asthmatic attacks in sensitized guinea pigs which resemble human bronchial asthma. In 1927 Ratner[4] introduced a method for sensitizing and inducing anaphylaxis in guinea pigs solely through the inhalation of dry antigenic dust. He called the symptom complex produced "experimental asthma" because it was comparable to the asthmatic syndrome in the human being. Kallos and Pagel[2] concluded from their work that finely sprayed antigen produces attacks in sensitized guinea pigs that clinically, roentgenogically, pharmacologically, and immunologically resemble human bronchial asthma.

The primary pulmonary change in asthmatic breathing is bronchiolar obstruction which in turn produces the signs and symptoms.[5] There have been relatively few studies, however, which have considered the pulmonary physiology of experimental asthma in the guinea pig. Recently Amdur and Mead[6] have developed a technique which permits the quantitative measurement of airway obstruction and of changes in the elastic properties of the lungs in unanesthetized guinea pigs. The present report deals with the investigation of the mechanical properties of the lungs in experimental asthma in the guinea pig.

METHOD

Measurement of Respiratory Variables.—The method for measuring the mechanical properties of the lungs of guinea pigs is based on the technique of Amdur and Mead.[6] The three variables which are necessary to calculate the mechanical properties of the lungs are intrapleural pressure, tidal volume, and rate of flow of air in and out of the respiratory system.

Intrapleural pressure was measured by inserting a fluid-filled polyethylene catheter directly into the chest cavity with the animal under light ether anesthesia. The animals recovered rapidly from the anesthesia and showed no signs of discomfort from the presence of the catheter. In about 10 per cent

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of the animals, the catheter nicked the lung. The data from these animals did not show a significant difference from the others. The ends of the catheter were connected through adapters to three-way stopcocks. This permitted the tube to be flushed regularly with a heparin-saline solution (2.0 per cent). For pressure recording, a Technitrol Lilly manometer, and a Grass driver amplifier and oscillograph were employed.

Tidal volume was measured by recording the pressure changes produced in a body plethysmograph with the animal's head protruding outside of the chamber. The pressure changes of the system were transmitted to a Statham transducer, Sanborn carrier amplifier, and a Grass oscillograph.

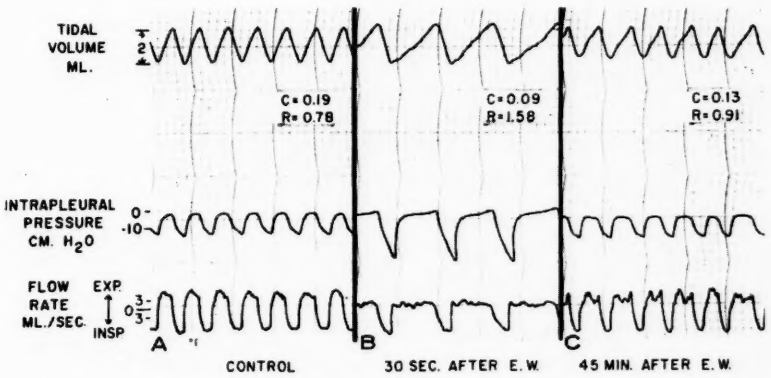

Fig. 1.—Sample tracings of guinea pig respiration. *A*, Tracing made during a control period. *B*, Tracing from the same sensitized animal thirty seconds after exposure to egg white. *C*, Tracing recorded forty-five minutes after exposure.

The rates of the flow of air in and out of the respiratory system were obtained by electrical differentiation of the volume signal with respect to time. A tracing of the flow pattern was obtained by feeding the flow rate signal into a Grass amplifier and oscillograph. The final calculations were made from the recorded tracings. A sample tracing is shown in Fig. 1.

By relating the volume and the flow rate to intrapleural pressure at specific points during the respiratory cycle, information on the mechanical properties of the lungs can be obtained. The intrapleural pressure has an elastic component and a flow resistive component. The elastic component relates to the elastic recoil of the lungs. The flow resistive component relates to resistance to flow of gas in the lungs and airway and to viscous resistance of the lung tissues. The elastic component and flow resistive component of the intrapleural pressure are evaluated separately.

At the beginning and end of inspiration no air is being moved into or out of the respiratory system, hence, at these moments the flow resistive component drops out, and any pressure change and the accompanying volume change must relate to elastic forces only. The volume change per unit of pressure change at instants of no flow is called compliance, which is a measure of the elastic distensibility of the lungs (Fig. 2).

At points of equal volume during the respiratory cycle the elastic forces must be approximately equal, and the pressure change between two such points must relate chiefly to resistance to flow in the lungs and the airway. This pressure change is related to the associated flow change to give a measure of the flow resistance of the lungs and airway. This value represents the average inspiratory and expiratory resistance near peak inspiratory and expiratory flows (Fig. 2).

The present study was divided into two parts.

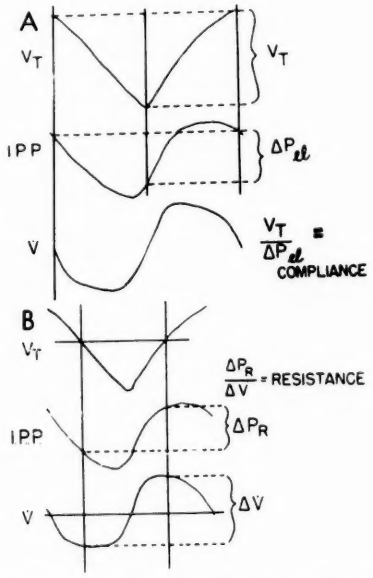

Fig. 2.—Method of measuring pulmonary compliance (*A*) and pulmonary resistance (*B*). (From Amdur and Mead.[8])

Part I.—A series of ten male guinea pigs, weighing 210-270 grams, were individually placed in the body plethysmorgraph, and a small exposure chamber was attached. Control records were taken under this condition with each animal serving as its own control. The chamber had an aperture for a No. 40 DeVilbiss nebulizer which was attached to a compressed air tank delivering 10 L. of air per minute. The nebulizer served to introduce a fine mist of histamine phosphate (1:1000) with the particle size distribution extending from 0.3 micron to 2 micra.[7] The histamine was used in order to investigate the effect of a known bronchoconstrictor on resistance and compliance. The animals were exposed to histamine until they developed signs of shock with the duration of exposure varying between one and four minutes. Records were taken of respiratory functions every minute during the exposure and at five-minute intervals for thirty minutes.

Part II.—Twenty-seven male guinea pigs, weighing 220-280 grams, were injected intraperitoneally three times with 0.25 ml. of fresh, undiluted egg white in a one-week period. Following the series of injections two weeks were

allowed to elapse for sensitization to occur. At this time the guinea pigs were exposed to a dilute solution of egg white (1:20 in saline) aerosolized by the DeVilbiss nebulizer. The duration of exposure was ten minutes, except in the case of three animals who developed anaphylactic shock within the first few minutes. Records were taken of respiratory functions every minute during the exposure and at five-minute intervals for ninety minutes. Each animal served as its own control.

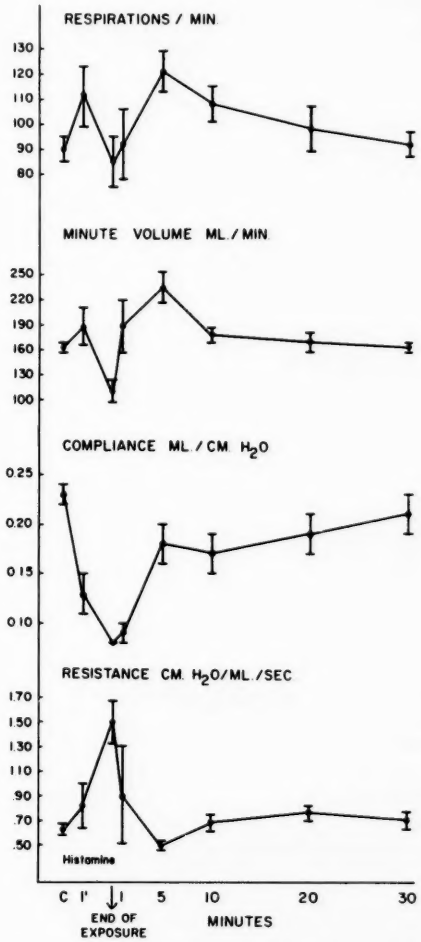

Fig. 3.—Effect of exposure to histamine. Values expressed as mean ± standard error (n=10). *C* refers to control measures. The period of exposure to histamine varied between one and four minutes.

RESULTS

Part I.—The respiratory responses to histamine during and following exposure can be seen in Fig. 3 and Table I. The exposure to histamine resulted in a significant decrease in minute volume ($p < 0.001$), increase in resistance ($p < 0.001$), and decrease in compliance ($p < 0.001$). There were no significant changes in respiratory rate. One minute after the termination of the

histamine mist the minute volume and resistance did not differ significantly from the control values. The decrease in compliance, however, persisted for twenty minutes after the cessation of histamine.

Part II.—The twenty-seven sensitized animals showed a wide range of individual variation in their reaction to the antigen. Seven guinea pigs were nonreactive without any significant changes in the ventilatory data or in resistance or compliance. Seventeen animals in the sensitized group reacted

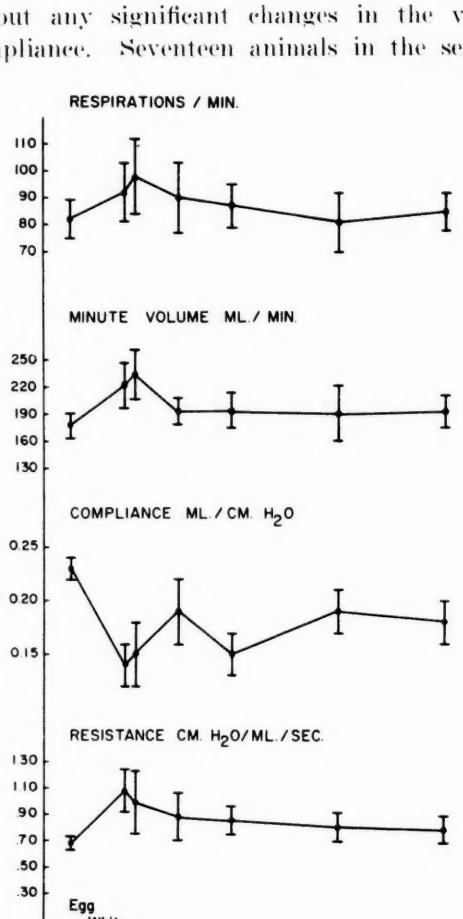

Fig. 4.—Effect of exposure to egg white in previously sensitized animals. Values expressed as mean ± standard error (n=8). C refers to control measures. The animals were exposed to the egg white spray for ten minutes.

to the antigen with the clinical signs of experimental asthma. In the majority of these animals, inspiration was shortened while expiration was markedly prolonged (Fig. 1). In nine of the seventeen animals who reacted to the antigen, the expiratory side of the respiratory pattern of tidal volume was not a smooth curve and did not permit the measurement of resistance with the technique utilized in this study. These animals, therefore, were not included in further analysis although they showed a significant decrease in compliance.

TABLE I. EFFECTS OF HISTAMINE ON RESPIRATION

RESPIRATORY VARIABLES	CONTROL	END OF EXPOSURE	+ 1 MIN.	+ 5 MIN.	+ 10 MIN.	+ 20 MIN.	+ 30 MIN.
Respirations per minute	90 ± 5*	85 ± 10	92 ± 14	121 ± 8	108 ± 7	98 ± 9	92 ± 5
Minute volume (ml.)	164 ± 6	111 ± 14†	190 ± 31	234 ± 18‡	177 ± 9	169 ± 13	163 ± 7
Compliance (ml./cm. H_2O)	0.23 ± 0.01	0.03 ± 0.01†	0.09 ± 0.01†	0.18 ± 0.02§	0.17 ± 0.02‖	0.19 ± 0.02§	0.21 ± 0.02
Resistance (cm. H_2O/ml./sec.)	0.63 ± 0.02	1.54 ± 0.17†	0.91 ± 0.40	0.50 ± 0.04	0.68 ± 0.07	0.76 ± 0.06	0.70 ± 0.07

*All values expressed as mean ± standard error (n = 10).
†Differs significantly from control p = 0.001.
‡Differs significantly from control p = 0.01.
§Differs significantly from control p = 0.05.
‖Differs significantly from control p = 0.02.

The course of the remaining eight animals who reacted to the antigen with experimental asthma can be seen in Fig. 4 and Table II. Following exposure to the egg white mist there was a significant increase in minute volume (p 0.01), decrease in compliance ($p < 0.001$), and increase in resistance (p 0.02). There were no significant changes in respiratory rate. The minute volume and resistance returned almost immediately to the level of the control values. The decrease in compliance persisted for thirty minutes.

TABLE II. RESPIRATORY RESPONSE IN EXPERIMENTAL ALLERGIC ASTHMA

RESPIRATORY VARIABLES	CONTROL	END OF EXPOSURE	+ 1 MIN.	+ 5 MIN.	+10 MIN.	+ 20 MIN.	+ 30 MIN.
Respirations per minute	82 ± 7*	92 ± 11	98 ± 14	90 ± 13	87 ± 8	81 ± 11	85 ± 7
Minute volume (ml.)	177 ± 14	222 ± 25†	235 ± 28§	194 ± 15	194 ± 20	191 ± 31	193 ± 18
Compliance (ml./cm. H_2O)	0.23 ± 0.01	0.14 ± 0.02‡	0.15 ± 0.03§	0.19 ± 0.03	0.15 ± 0.02§	0.19 ± 0.02‖	0.18 ± 0.02†
Resistance (cm. H_2O/ml./sec.)	0.68 ± 0.05	1.08 ± 0.16§	0.99 ± 0.24	0.88 ± 0.18	0.85 ± 0.11	0.80 ± 0.11	0.78 ± 0.10

*All values expressed as mean ± standard error (n=8).
†Differs significantly from control p = 0.01.
‡Differs significantly from control p = 0.001.
§Differs significantly from control p = 0.02.
‖Differs significantly from control p = 0.05.

Three of the animals in the sensitized group developed clinical signs of anaphylactic shock within a period of one to three minutes, and one of the animals died. These guinea pigs showed a marked decrease in respiratory rate and minute volume, and a marked increase in resistance and decrease in compliance (Table III).

TABLE III. RESPIRATORY RESPONSE IN ANAPHYLACTIC SHOCK

TIME	RATE (RESP./MIN.)	TIDAL VOLUME (ML.)	MINUTE VOLUME (ML./MIN.)	RESISTANCE (CM. H_2O/ ML./SEC.)	COMPLIANCE (ML./CM. H_2O)
Control	80*	2.7	206	0.61	0.30
End of exposure	40	2.5	84	1.05	0.09
Per cent change	−50	−7.0	−59	+72.00	−70.00

*All values expressed as mean (n=3).

DISCUSSION

The findings of the present study show that the changes in the mechanical properties of the lungs of guinea pigs in experimental asthma are an increase in resistance and decrease in compliance. Similar changes also are observed following exposure to histamine. In contrast to relatively uniform responses to histamine, there are considerable individual differences among guinea pigs in the development of experimental allergic asthma.

Approximately 25 per cent of the animals exposed to egg white were nonreactive without significant changes in resistance or compliance. The question might be raised whether these animals were sensitized, since Ratner[1] has reported that guinea pigs vary in their susceptibility to sensitization. He reported that 7 per cent of his animals were resistant to sensitization despite repeated exposures. Some of the animals in the nonreactive group in the present study, therefore, may not have been sensitized. Schiavi and Stein[5] found, however, that 100 per cent of a group of thirty animals sensitized in the manner reported herein developed signs of anaphylactic shock and died when challenged with an intracardiac injection of egg white.

The failure of the nonreactive animals to develop asthma may be related to the ether which was administered prior to exposure to the antigen. Several investigators[9, 10] have reported that ether anesthesia provides a protective action against anaphylaxis, while others[11] have not substantiated this observation. It has been found, however, that ether as administered in this study did not have any significant effect upon the clinical signs or upon the respiratory rate, tidal volume, or inspiratory-expiratory flow ratio in experimental allergic asthma.[5] It is possible that the failure of some of the animals to develop asthma may be related to a lack of responsiveness of the respiratory system to an antigen-antibody reaction. This requires further investigation.

Within the group of animals that reacted to the egg white mist, there was considerable variation in response which ranged from mild asthma to lethal anaphylactic shock. In the animals that developed experimental allergic asthma there was a significant increase in resistance and decrease in compliance. These findings are similar to those reported by Noelpp and Noelpp[3] utilizing a different technique for measuring resistance and compliance in the guinea pig. In the three guinea pigs that developed anaphylactic shock in the present study, the changes in the respiratory variables were of the same order as observed following exposure to histamine.

Ratner,[1] Kallos and Pagel,[2] and others[3] have reported that the administration of a homologous antigen to previously sensitized guinea pigs results in unequivocal hypersensitive reactions of varying degrees of intensity. These reactions are clinically and immunologically similar to human bronchial asthma. McIlroy and Marshall[12] have shown that the two main abnormalities in the mechanical properties of the lungs in human asthma are an increase in resistance to the flow of air in and out of the lungs and a decrease in compliance. The findings of the present report demonstrate that the mechanics of breathing in experimental allergic asthma are similar to human bronchial asthma.

It has been reported by Ottenberg, Stein, and others[13] that a respiratory syndrome resembling asthma can be conditioned in the guinea pig. They raised the question, however, whether the conditioned attacks were analogous to experimental allergic asthma. The procedure reported herein has considerable importance for further studies of conditioned asthma and should determine whether conditioned asthma in the guinea pig is physiologically similar to experimental allergic asthma, as well as human bronchial asthma.

SUMMARY

1. A technique has been described which permits the quantitative measurement of the resistance to air flow and compliance in experimental asthma in the guinea pig.

2. Animals exposed to histamine reacted uniformly with a significant increase in resistance and decrease in compliance.

3. In contrast, there was a wide range of variation in the changes in resistance and compliance of the sensitized animals exposed to the antigen. The reactivity varied from no response to marked changes in the mechanics of breathing in lethal anaphylactic shock.

4. The mechanics of breathing in experimental allergic asthma are similar to human bronchial asthma.

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ASTHMA DUE TO PARAKEET AND CANARY FEATHERS

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AFTER the removal of restrictions upon the importation of psittacine birds into the United States, there has been a great increase in their popularity. It has been estimated that one home in five in this country now has a parakeet.[1] During the last year we have studied the reactions of five patients whose asthma was markedly exacerbated by exposure to either parakeet or canary feathers.

A careful review of the literature revealed only three case reports of sensitivity to parrots, all written in the 1920's, and one report of allergy to canaries. Walker,[2] in 1920, described a 17-year-old boy with marked asthma due to feather sensitivity who did not become well on feather control but who improved rapidly when his pet parrot was removed. Piness[3] reported the case of a man whose first attack of asthma had occurred one year after he had obtained three parrots, and who wheezed only while at home. Skin tests revealed strongly positive reactions to parrot and chicken feathers. Removal of his feather pillow and his parrot resulted in complete elimination of symptoms. Brown[4] reported a man who developed coryza and rhinorrhea after obtaining a parrot. His only positive skin test was to parrot feathers and upon removal of the parrot the symptoms disappeared. Unger[5] described an asthmatic old man who lived in a shack with fifty canaries. He had a markedly positive skin test with canary feather extract but refused to give up his occupation of breeding canaries. Hyposensitization to canary extract was unsuccessful.

MATERIALS AND METHODS

Extracts.—These were aqueous solutions prepared in this laboratory by extraction with Coca's extracting fluid for forty-eight hours at 4° C., dialyzed and concentrated to the osmolality and pH of buffered saline. They were then standardized in protein nitrogen units per milliliter (PNU*), according to the method of Cooke and Stull.[6] The feather extract used routinely in this clinic, designated in the text as "mixed down-feathers," but in the tables as "feathers," was prepared from a mixture of equal parts of chicken, duck, and goose feathers and defatted with carbon tetrachloride. The extract of parakeet feathers was similarly prepared. The canary feather extract was obtained from Hollister-Stier Laboratory but was standardized in protein nitrogen units in

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*One protein nitrogen unit (PNU) equals 0.00001 mg. of phosphotungstic precipitable nitrogen.

our laboratory. The buffered saline diluting fluid contained in one liter follows: 0.363 Gm. potassium phosphate; 0.431 Gm. sodium phosphate (Na_2PO_4-$12H_2O$); 5.0 Gm. sodium chloride; 4.0 Gm. phenol, and a sufficient quantity of distilled water.

Direct Skin Tests.—One fortieth of a milliliter of the indicated extract was injected intracutaneously on the upper arm in an area not previously used for injection therapy. Fifteen minutes later the reactions were read as follows: negative (Neg.), no demonstrable reaction; equivocal (±), questionable positive; slight (Slt.), definite wheal of 3 to 7 mm. diameter; moderate (Mod.), 8-12 mm. wheal; marked (Mkd.), greater than 12 mm. wheal, or with pseudopods.

Passive-Transfer Studies.—One tenth of a milliliter of the patient's serum was injected intracutaneously on the back of a nonallergic recipient. For dilution tests, the serum was mixed with the required amount of buffered saline diluting fluid and 0.1 ml. of the dilution used to sensitize each site. Forty-eight hours after placing, the sites were tested with one twentieth of a milliliter of the 1,000 PNU concentration of each of the three types of feather extract. Reactions were read fifteen minutes later as follows: negative (Neg.), wheal less than 3 mm. in diameter; equivocal, (±) 3 mm. wheal; 1+, 4 to 6 mm. wheal; 2+, 7 to 9 mm. wheal; 3+, 10 to 12 mm. wheal; 4+ greater than 12 mm. wheal, or with pseudopods.

Neutralization Studies.—Equal parts of the patient's serum were mixed with each of the three feather extracts, 500 PNU or 4,000 PNU as specified in the text and tables, as well as a control of buffered saline. These were then placed overnight in the refrigerator. Then 0.1 ml. of each mixture was injected intracutaneously into each of several sites on the back of a nonallergic recipient and the reaction noted. Forty-eight hours later each of the sites were retested with one of the feather extracts in 1,000 PNU concentration. The reactions were graded as described for passive transfer.

CASE REPORTS

CASE 1.—V. A., a 39-year-old Puerto Rican born woman, was admitted to Roosevelt Hospital for the first time on June 1, 1959, because of asthma which had been very severe for two and a half years prior to admission, following bronchopneumonia associated with the birth of her first child. During the next ten years, asthma had occurred very infrequently and was usually associated with respiratory infections. Three years prior to admission, her husband had purchased two parakeets. About three months later she began to have repeated severe attacks of asthma. This was perennial, but worse during the spring.

On admission she was in acute respiratory distress. Physical examination revealed bilateral expiratory wheezes and pale boggy nasal mucosa, but no other abnormalities. Chest x-ray was normal. Sinus x-rays showed cloudiness of both maxillary antra due to mucous membrane thickening. Intracutaneous tests gave moderately positive reactions to undiluted house dust, grass 1,000 PNU/ml., 5-tree mixture* 1,000 PNU/ml., and mixed feathers. There was a marked reaction to ragweed 1,000 PNU/ml. All other inhalant skin tests were negative.

Upon treatment with Tedral, steroids, and epinephrine, the symptoms cleared rapidly. She was discharged home asymptomatic on June 12, 1959.

*The 5-tree mixture contains equal parts of ash, birch, hickory, oak, and poplar pollen extracts.

Second admission.—Within several hours of her arrival at home she began to wheeze and was readmitted to the hospital on June 19, 1959. There was a rapid recovery. After an intracutaneous injection of 40 PNU of grass she had a mild constitutional reaction. Skin tests performed on July 7, 1959, revealed a marked reaction to parakeet feather extract 1,000 PNU/ml. (see Table I). She was discharged home asymptomatic on July 9, 1959, with instructions to get rid of the parakeets.

Third admission.—Within several hours of arriving home she again started to wheeze, and was readmitted to the hospital on July 12, 1959. This time she was not discharged until her husband had given away the parakeets and thoroughly vacuum-cleaned their apartment.

During the first two weeks after her discharge on July 24, 1959, she had several mild episodes of asthma readily controlled with Tedral and 10 mg. daily of prednisone. Until May, 1960, she had had no asthma attacks. She feels so well that it has been difficult to convince her to come regularly to the clinic for desensitization injections.

Serum was obtained from this patient for passive-transfer neutralization studies (Table I). Direct skin tests had shown a more marked response to the parakeet extract than to the mixed down-feather extract. On two passive-transfer tests, her serum gave a positive reaction to parakeet feather extract at a 1-30 dilution, but gave unequivocally positive reactions to the mixed down-feathers and the canary feathers only with undiluted serum.

TABLE IA. DIRECT SKIN TESTS AND DILUTION STUDIES (CASE 1)

TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU	FEATHERS 100 PNU	PARAKEET 100 PNU
Direct	Moderate	Marked	—*	Slight	Moderate
Passive transfer No. 1					
Undiluted serum	1+	4+	—	—	—
1-10 dilution	Negative	3+	—	—	—
1-100 dilution	Negative	Negative	—	—	—
Passive transfer No. 2					
Undiluted serum	1+	4+	1+	—	—
1-3 dilution	Negative	3+	±	—	—
1-10 dilution	Negative	2+	Negative	—	—
1-100 dilution	Negative	1+	Negative	—	—

*Not done.

TABLE IB. NEUTRALIZATION STUDIES

ANTIGEN ADDED TO PATIENT'S SERUM	INITIAL REACTION	TESTING SOLUTIONS		
		FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU
Feathers 4,000 PNU	4+	1+	1+	1+
Parakeet 4,000 PNU	3+	±	Negative	2+
Canary 4,000 PNU	2+	2+	±	Negative
Buffered saline	Negative	1+	3+	3+
Recipient's reaction	Negative	1+	Negative	Negative

Neutralization studies were performed with 5,000 PNU/ml. of each type of feather extract (Table IB). The site prepared with a mixture of serum and mixed down-feathers gave a negative reaction on retesting with the same extract (1+ reaction was the same as the control for down-feathers), but positive reactions to parakeet and canary feather extracts. The parakeet feathers completely neutralized the reaction to both the mixed down-feather extract and the parakeet feather extract, but not to the canary extract. The canary feather extract completely neutralized only to the canary extract.

We interpret these studies as indicating that she was sensitive primarily to parakeet feathers, with a degree of cross-sensitivity to antigens found in down-feathers. The canary feathers appeared to contain a separate antigen.

CASE 2.—E. C., a 37-year-old white woman, was first seen in our clinic in August, 1959, because of asthma of five months' duration. She had been suffering from constant rhinorrhea in October, 1958. In March, 1959, nocturnal coughing and wheezing developed. This necessitated admission to another hospital in May, 1959. The asthma cleared rapidly during this hospital stay but recurred as soon as she arrived home. She had a foam rubber pillow, cotton mattress, and a parakeet which she had had for three years.

Skin tests showed a slight reaction to undilated house dust, equivocal reaction to mixed down-feathers 1,000 PNU/ml., and a moderate reaction to parakeet feathers 1,000 PNU/ml. (Table II). All other skin tests were negative. Sinus x-rays showed slight antral clouding.

TABLE II. STUDIES ON CASE 2

TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU
Direct	±	Moderate	—*
Passive transfer No. 1			
Undiluted serum	1+	2+	—
Passive transfer No. 2			
Undiluted serum	±	1+	Negative
1-3 dilution	Negative	Negative	Negative

*Not done.

In late August, 1959, she gave away her parakeet, and for eleven days there were only two slight attacks of asthma. However, when the bird was returned she immediately developed severe wheezing which persisted for one and a half hours, until the bird was again removed.

As of May, 1960, she had had occasional mild attacks of asthma, not severe enough to necessitate loss of time from work, and had been receiving injections of house dust and bacterial vaccine.

Two passive-transfer tests revealed more marked reactions to the parakeet feathers than to the mixed down-feathers. Of the sixty-four patients in whom comparative skin tests with parakeet feathers and mixed down-feathers have been performed, only this case and Case 1 have shown such a reaction which, we believe, strongly suggests clinical allergy to parakeets.

CASE 3.—A. R., a 42-year-old white woman, was first seen in this allergy clinic in 1955 because of urticaria. There was a history of "bronchitis" for three years. She had a feather pillow. Physical examination at that time revealed scattered wheezes in both lung fields. Intradermal skin tests showed marked reactions to undiluted house dust and mixed down-feathers 1,000 PNU/ml., and slight reactions to ragweed 100 PNU/ml., and pyrethrum 1,000 PNU/ml. Tests for all other inhalants were negative. She was given injections of house dust and stock bacterial vaccine, but continued to have occasional asthma attacks. In July, 1957, while on a trip to Europe, she was free of asthma but immediately began to wheeze upon her return.

Requestioning in January, 1960, revealed that she had had a parakeet for three years. An intracutaneous test showed a marked reaction to parakeet feathers 1,000 PNU/ml. (Table III). From the time of removal of the parakeet until May, 1960, she had had a very marked decrease in the frequency of her asthma attacks.

Passive-transfer studies (Table III) showed a slightly more marked reaction to the mixed down-feathers than to the parakeet feathers. Neutralization studies were performed with 500 PNU/ml. of each type of feather extract. The mixed down-feather extract neutralized completely the reaction to both parakeet feather and mixed down-feather extracts; the parakeet feather extract neutralized only to the parakeet feather extract; and the canary feather extract neutralized to neither. We interpret these studies as showing that this patient is primarily sensitive to down-feathers, but also has antibodies to some antigens of mixed down-feathers found also in parakeet feathers.

TABLE IIIA. DIRECT SKIN TESTS AND DILUTION STUDIES (CASE 3)

TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU	FEATHERS 100 PNU	PARAKEET 100 PNU
Direct	Marked	Marked	—*	Moderate	Moderate
Passive transfer No. 1					
Undiluted serum	3+	1+	—	—	—
Passive transfer No. 2					
Undiluted serum	2+	1+	1+	—	—
1-10 dilution	Negative	Negative	Negative	—	—

*Not done.

TABLE IIIB. NEUTRALIZATION STUDIES

ANTIGEN ADDED TO PATIENT'S SERUM	INITIAL REACTION	TESTING SOLUTIONS		
		FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU
Feathers 500 PNU	3+	Negative	Negative	Negative
Parakeet 500 PNU	Negative	2+	Negative	Negative
Canary 500 PNU	Negative	2+	1+	Negative
Buffered saline	Negative	3+	1+	Negative

CASE 4.—C. D., a 65-year-old Puerto Rican woman, was first seen in this allergy clinic on March 4, 1960, because of asthma of ten years' duration. This was worse in the evening and was associated with rhinorrhea and sneezing. The symptoms were perennial with some increase in severity during the winter. She had a feather pillow and no pets at home. However, her married daughter had 2 parakeets and her frequent exposure to these birds almost invariably brought on an attack of asthma.

On physical examination she had a pale wet nasal mucosa. The lungs were clear. Chest x-ray was normal. Sinus x-rays revealed a large polyp in the left maxillary antrum, with moderate mucosal thickening in the right antrum. The white cell count was 13,500 with 29 per cent eosinophils.

Intracutaneous tests showed a marked reaction to undiluted house dust extract, moderate reaction to mixed down-feather extract 1,000 PNU/ml., and slight reactions to parakeet feather extract 1,000 PNU/ml., and canary feather extract 1,000 PNU/ml. (Table IV). Skin tests with other common inhalants gave negative results.

Upon removal of the feather pillow, she had had almost complete clearing of her asthma.

TABLE IV. STUDIES ON CASE 4

TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU
Direct	Moderate	Slight	Slight
Passive transfer			
Undiluted serum	2+	1+	Negative
1-3 dilution	1+	Negative	Negative
1-10 dilution	Negative	—*	—

*Not done.

Passive-transfer studies (Table IV) showed her reactions to be slightly more marked to mixed down-feathers than to the parakeet feathers and negative to canary feathers. Although she is more highly sensitive on skin testing to mixed down-feathers than to parakeet feathers, there is excellent clinical and skin test evidence that she is also sensitive to parakeet feathers.

CASE 5.—G. M., a 33-year-old Puerto Rican born woman, was first seen in this allergy clinic on Nov. 10, 1958, because of asthma of fifteen years' duration, worse for the previous

eight months. Asthma had first occurred at the age of 18 years, during her first pregnancy, but attacks had been very infrequent. Eight months prior to her first clinic visit her husband had bought a canary. Since then she had been suffering from daily asthmatic attacks. Skin tests during her first clinic visit showed a marked reaction to house dust and slight reaction to mixed feather extract 1,000 PNU/ml. All other common inhalant tests were negative. No test for canary feathers was done at that time.

She was followed in the allergy clinic from November, 1958, to July, 1959. Despite injections of house dust and bacterial vaccine, she had frequent episodes of asthma, requiring steroid therapy. From August, 1959, until January, 1960, she was in a mental hospital because of a "nervous breakdown." During her entire hospital stay, there were no asthma attacks. However, upon returning home in January, 1960, wheezing immediately recurred.

First Hospital Admission.—Because of the increase in her asthmatic symptoms she was admitted to Roosevelt Hospital on Feb. 5, 1960. Physical examination at that time revealed many wheezes throughout both lung fields, but was otherwise normal. Chest x-ray was normal. Sinus x-rays showed mucous membrane thickening in both antra. Skin tests showed marked reaction to canary feather extract 1,000 PNU/ml. (Table V). Upon treatment with epinephrine and Tedral there was rapid improvement and she was discharged home asymptomatic Feb. 16, 1960.

TABLE V. STUDIES ON CASE 5

TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU
Direct	Slight	Slight	Marked
Passive transfer No. 1			
Undiluted serum	Negative	Negative	2+
1-10 dilution	Negative	Negative	2+
1-100 dilution	—*	—	±
Passive transfer No. 2			
Undiluted serum	Negative	±	3+
1-3 dilution	Negative	±	—
1-10 dilution	—	—	2+
1-50 dilution	—	—	Negative

*Not done.

Second Admission.—Before her discharge her husband had been instructed to give away the canary and to thoroughly clean and vacuum the apartment. He followed only the first part of the instructions and did nothing to remove the residual canary feathers from their home. Within forty-eight hours of her return home she was suffering from severe asthma and within seventy-two hours she had to be readmitted in status asthmaticus. She was treated with intravenous aminophylline, triamcinolone, epinephrine, and intermittent positive pressure breathing. Medications were gradually reduced and, at the time of her discharge on March 1, 1960, she was completely clear of asthma and taking no medication.

From the time of her discharge until May, 1960, she had only one mild asthma attack, not requiring the presence of a physician. She feels so well that it has been difficult to get her to come regularly to the allergy clinic.

Two passive-transfer studies were performed with this patient's serum (Table V). Both times the serum gave a positive reaction to canary feather extract in 1-10 dilution, and negative or equivocal reactions to mixed down-feather and parakeet feather extracts, even with undiluted serums. The strongly positive reactions to canary feather extract and slight or negative reactions to the other feather extract suggest a rather specific reaction to canary feathers.

In none of these cases which we have presented can one say that parakeet or canary feathers was the *only* thing to which the patients were allergic. However, we feel that allergy to parakeet or canary feathers was a significant cause

of the asthmatic symptoms on exposure for the following reasons: (1) marked exacerbation of symptoms on exposure to the feathered pet, (2) prompt relief of symptoms after separation from the bird, and (3) demonstration of skin sensitizing antibody by both direct and passive-transfer testing.

STUDIES ON KNOWN FEATHER-SENSITIVE PATIENTS

In order to investigate further the relationship between the antigens in the various types of feathers we performed comparative skin tests on fifty-nine individuals known to be sensitive to mixed down-feathers. (The 5 patients whose histories have been presented were not included.) Every patient was tested with the same concentration of the extract of mixed down-feathers, parakeet feathers, and, in about half the cases, canary feathers (Table VI).

TABLE VI. COMPARATIVE SKIN REACTIONS OF 59 KNOWN FEATHER-SENSITIVE PATIENTS WHEN TESTED WITH PARAKEET AND CANARY FEATHER EXTRACTS

REACTION TO MIXED DOWN-FEATHERS	NUMBER OF PATIENTS	OTHER TESTING EXTRACT	NUMBER OF PATIENTS REACTING TO EACH TEST				
			MARKED	MODERATE	SLIGHT	NEGATIVE	NOT DONE
Marked to 1,000 PNU	8	Parakeet 1,000 PNU	1	2	3	2	0
		Canary 1,000 PNU	0	2	2	1	3
Moderate to 1,000 PNU	22	Parakeet 1,000 PNU	0	11	7	4	0
		Canary 1,000 PNU	0	7	4	4	7
Slight to 1,000 PNU	11	Parakeet 1,000 PNU	0	0	4	7	0
		Canary 1,000 PNU	0	0	1	4	6
Marked to 100 PNU	4	Parakeet 100 PNU	0	1	1	2	0
		Canary 100 PNU	0	1	1	1	1
Moderate to 100 PNU	11	Parakeet 100 PNU	0	3	6	2	0
		Canary 100 PNU	0	4	0	2	5
Slight to 100 PNU	3	Parakeet 100 PNU	0	0	2	1	0
		Canary 100 PNU	0	0	1	0	2
Totals	59		1	31	32	30	24

Every one gave a positive response to the mixed down-feather extract, forty-one gave a positive reaction to the parakeet feather extract, and eighteen gave negative reactions. To the canary feather extract there were twenty-three positive and twelve negative reactions.

However, not one of this group of people gave a reaction to either parakeet or canary feather extract stronger than their reaction to the mixed down-feather

extract (Table VI). Of all the individuals we have tested with these extracts to date, the only three who have shown greater reactivity to the individual feather extracts than to the mixed down-feather in the same concentration were Cases 1, 2, and 5. A reaction to the parakeet or canary feather extracts greater than to the mixed down-feathers in the same concentration appears to indicate sensitivity to the particular type of bird.

Passive-transfer studies were performed with the serum of seven of these patients known to be sensitive to down-feathers (Tables VII and VIII). Six of these patients showed positive reactions to mixed down-feathers on passive transfer, and one showed equivocal results. However, the serum of only one patient showed consistent, unequivocally positive reaction to the parakeet feather extract. This patient's reactions are summarized in Table VIII. On two dilution studies, her reaction to the mixed down-feather extract was slightly more marked than to the parakeet or canary extracts. Neutralization studies were performed with 4,000 PNU/ml. of each type of feather extract. The mixed down-feather extract completely neutralized the reaction on retesting with both the mixed down-feather extract and the parakeet feather extract. The parakeet feather extract neutralized only the reaction to parakeet feathers, and the canary feather extract did not neutralize any of the subsequent test reactions. Our conclusion is that this patient is allergic to an antigen or antigens found in many kinds of feathers, but present to a greater degree in the mixed down-feathers. In addition, she appears to react to something specific to canary feathers.

TABLE VII. RESULTS OF DIRECT SKIN TESTS AND PASSIVE TRANSFER ON SIX KNOWN FEATHER-SENSITIVE PATIENTS

PATIENT	TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU	FEATHERS 100 PNU	PARAKEET 100 PNU	CANARY 100 PNU
N. S.	Direct	Marked	Slight	Slight	—*	—	—
	Passive transfer No. 1†	2+	Negative	—	—	—	—
	Passive transfer No. 2†	1+	Negative	Negative	—	—	—
H. L.	Direct	Moderate	Negative	Negative	—	—	—
	Passive transfer No. 1	1+	Negative	—	—	—	—
	Passive transfer No. 2	1+	Negative	—	—	—	—
D. S.	Direct	Moderate	Moderate	—	—	—	—
	Passive transfer†	±	Negative	—	—	—	—
D. H.	Direct	—	—	—	Marked	Slight	—
	Passive transfer	1+	±	—	—	—	—
D. E.	Direct	Marked	Moderate	—	Moderate	Negative	Moderate
	Passive transfer No. 1	3+	1+	—	—	—	—
	Passive transfer No. 2	3+	Negative	1+	—	—	—
	1-10 dilution	2+	Negative	Negative	—	—	—
	1-100 dilution	±	—	—	—	—	—
E. S.	Direct	—	—	—	Marked	Negative	Negative
	Passive transfer	3+	Negative	Negative	—	—	—
	1-3 dilution	—	Negative	Negative	—	—	—
	1-10 dilution	2+	—	—	—	—	—
	1-100 dilution	±	—	—	—	—	—

*Not done.
†Unless otherwise specified in this table, undiluted serum was used.

TABLE VIIIA. DIRECT SKIN TESTS AND DILUTION STUDIES*

TYPE OF TEST	FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU	FEATHERS 100 PNU	PARAKEET 100 PNU	CANARY 100 PNU
Direct	—†	—	—	Marked	Moderate	Moderate
Passive transfer No. 1						
Undiluted serum	4+	3+	2+	—	—	—
1-10 dilution	2+	2+	1+	—	—	—
1-100 dilution	Negative	Negative	Negative	—	—	—
Passive transfer No. 2						
Undiluted serum	3+	2+	2+	—	—	—
1-3 dilution	1+	1+	1+	—	—	—
1-10 dilution	1+	Negative	Negative	—	—	—
1-30 dilution	Negative	Negative	Negative	—	—	—

*Studies on a patient who gave strong reactions to all three types of feather extract.
†Not done.

TABLE VIIIB. NEUTRALIZATION STUDIES*

ANTIGEN ADDED TO PATIENT'S SERUM	INITIAL REACTION	TESTING SOLUTIONS		
		FEATHERS 1,000 PNU	PARAKEET 1,000 PNU	CANARY 1,000 PNU
Feathers 4,000 PNU	4+	Negative	Negative	1+
Parakeet 4,000 PNU	2+	1+	Negative	1+
Canary 4,000 PNU	1+	2+	Negative	1+
Buffered saline	Negative	2+	2+	1+

*Studies on a patient who gave strong reactions to all three types of feather extract.

SUMMARY AND CONCLUSIONS

In recent years there has been a marked increase in the parakeet population of the United States. Recently we observed five patients, hypersensitive to either parakeet or canary feathers. Each had a history of asthma after exposure to their feathered pet with relief of symptoms on separation from the pet, as well as positive direct skin tests and passive-transfer tests. In order to study further the relation of allergy to mixed down-feathers, parakeet feathers, and canary feathers, comparative skin tests were performed on fifty-nine clinic patients known to be sensitive to mixed down-feather extract. About two thirds of the reactions to the pet feather extracts were positive and one third were negative. However, there was no reaction to the parakeet or canary feather extract greater than the reaction of the same person to the mixed down-feather extract. We conclude that a reaction to the specific pet feather extract greater than to the mixed-down feather extract (of the same PNU concentration) is good evidence of specific sensitivity. Neutralization studies, performed on the serums of three patients, suggested the presence of several antigens, some present in only one species, others in all types of feathers.

The presence of parakeets, parrots, or canaries should be inquired about in all allergic histories. It is preferable that allergic individuals have no feathered pets in their home, just as it is preferable that they have no cats or dogs in their home. Removal of a pet bird from the household may at times produce dramatic improvement of a very troublesome case of asthma.

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A STUDY OF THE AIR-BORNE ALLERGENS OF THE VIRGIN ISLANDS NATIONAL PARK AND ADJACENT PARTS OF ST. JOHN ISLAND

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With the cooperation of Mr. Harold A. Hubler, then Superintendent of the Virgin Islands National Park, and the cooperation of Mr. Leslie H. Moore, Jr., and his technical staff of Caneel Bay Plantation, a pollen survey of this, the newest national park, and the privately owned areas of St. John Island was set up in the fall of 1958. As in the case of most of the other national parks, this study was to consist of both a careful examination of the flora of the Island and continuous sampling of the air for allergenic pollens and spores over a period of one year. The survey was inaugurated on October 23.

The Virgin Islands National Park encompasses two thirds of St. John, smallest of the three main U. S. Virgin Islands. The Park was established in 1956 as the result of the gift of land by Mr. Laurance S. Rockefeller and Jackson Hole Preserve, Incorporated. St. John is 75 miles east of Puerto Rico, three miles from St. Thomas, and is noted for its natural beauty, climate, and beaches. It is some nine miles long and five miles wide, has a population of about 800 persons, and is mountainous and heavily forested.

Our field studies were directed specially toward learning whether any species of ragweed (*Ambrosiaceae*) was to be found growing anywhere on the island. While carrying on this search, ample opportunity was afforded for detecting any other source of pollens which might later prove to be allergenic, particularly wind-borne pollens. As a part of the field work done during late October, Mr. Fafalla made a list of more than eighty native and exotic plants found on the Plantation and on adjacent Park areas and which were in flower at that time. Samples of pollen were collected from each of these for use in identification of pollens that might be caught subsequently in the course of the continuous air sampling.

Nearly two weeks were spent in an intensive search of the shore areas of St. John, particularly that along the numerous bays and the small neglected spots and gardens in the village of Cruz Bay, as well as the privately-owned farms outside of the Park area. Careful attention was also given to the lush tropical vegetation of the mountainous central part of the island and to the well-groomed open acreage of Caneel Bay Plantation.

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Our air sampler at the Plantation was placed on the flat roof of the open dining terrace at a distance of about 10 feet above ground and 30 feet from the bay shore. Here oil-coated slides were to be exposed throughout the ensuing year, changes of slides being made every two or three days. Mr. Fafalla assumed the task of identification and counting of pollen grain and mold spores found on the unbroken series of samples through the year. This location seemed to be ideal because of the prevailing winds that sweep down from the high central part of the island.

Not one ragweed plant of any species was found anywhere on the island. In view of the fact that two ragweed species in very small quantities had been noted on St. Thomas and a sizeable ragweed-contaminated area had been discovered by the senior author on Puerto Rico some seven years previously, it was thought possible that stray specimens of ragweed might be present on St. John. Two species of garden and wasteland weeds, *Ambrosia dubious* and *Parthenium hysterophorus*, common in parts of continental U. S. A. but of slight significance even there, were found in Cruz Bay village. There were not enough of these plants present to excite allergic symptoms, even though a person sensitized to them should reside continuously in the immediate area of the plants, much less for the few specimens to distribute effective quantities of pollen over the island. No pollen grains from any of the above species were found on the slides at Caneel Bay.

Of the trees and shrubs on St. John, none was found to be wind pollinated and none represented species that are known to cause even occasional allergy symptoms in the continental U. S. A. However, several insect-pollinated St. John species have been reported by allergists practicing elsewhere in the West Indies to be occasional causes of sensitization among residents of the islands. The mango tree, which is present on St. John, is an example of these. Of the tree and shrub pollen grains caught in the air sampling throughout the year, 60 per cent were from the half dozen coconut trees standing within 10 to 30 feet of the sampler and whose flowering spikes hung considerably above the slides. Coconut pollen has never been proved to be allergenic. The total catch of pollen from the nine other species of woody plants averaged considerably less than one pollen grain per unit slide area per day, which is certainly a negligible amount even if these pollen were known to be actually allergenic. In any case the tropical plants whose few pollen grains were caught need not cause any concern to visitors or guests on the island, who stay only a few hours or at most a few weeks and who seldom or never have encountered these pollens before. It is the experience of allergists that it usually requires several seasons of continuous, heavy exposure to unfamiliar types of pollen in order to induce sensitiveness to them.

It will be seen from Table I that the common wind-borne mold spores, which are a frequent cause of allergy in the middle and eastern states, are almost absent from the air of St. John, at least at Caneel Bay.

Thus the native and imported grasses seem to be the only possible sources of air-borne allergens on St. John. In the wooded areas which constitute a

TABLE 1. TOTAL POLLEN AND MOLD SPORE RECORD FOR ONE YEAR (NOV. 1, 1958, TO NOV. 1, 1959) IN GRAINS CAUGHT ON 1 SQ. CM. OF HORIZONTALLY-EXPOSED, OIL-COATED SLIDE

Pollens	
Grass (all species)	194
Coconut palm	229
Calabash, *Crescentia cujete*	1
Buttonwood, *Conocarpus erecta*	7
Genip, *Melicocca bijuga*	27
Turpentine tree, *Bursera simaruba*	18
Hog plum, *Spondias lutea*	1
Sea grape, *Coccolobis uvifera*	25
Gre-gre, *Bucida buceras*	14
Zanthoxylum	2
Scotch pine, *Pinus sylvestris*	1
Composite	3
Ragweed	0
Unidentified pollen	13
Spores	
Alternaria	2
Helminthosporium	9
Brachysporium	7
Rust	2
Unidentified spores	2

large portion of the island, grasses are crowded out by stronger types of vegetation. On the Plantation and in other cleared areas, various species of grass flourish. Bermuda grass is a favorite lawn grass on the Plantation. Elephant grass *(Pennesetum purpureum)* and various species of *Chloris* are common on other open areas.

Only during the month of May did the slides catch an average of as much as one pollen grain per day per square centimeter. Compared with the average catch per day during the grass season in many places in the central and eastern states, this represents a very low atmospheric concentration. One of the possible reasons for the low figure on St. John is that there is very little grass growing in the wooded parts of the island. The frequent rains and continuous high humidity are doubtless effective in curtailing grass pollen dispersal. But on the Plantation where there are several acreas of grassy area it would seem that frequent cutting must be partly responsible for low pollen production.

From the evidence we have gathered, the exposure to any kind of allergenic pollen and mold spores of visitors touring the Virgin Islands National Park at any time of the year is very small or negligible, and the exposure to ragweed pollen, zero.

IN VITRO LEUKOCYTOLYSIS IN PRESENCE OF POLLEN AND HOUSE DUST ANTIGENS

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THE EFFECT on leukocytes of adding antigen to the blood of allergic subjects has aroused periodic interest. Previous reports have reached varying conclusions. For example, Squire and Lee[1] found a significant drop in the total number of leukocytes when ragweed antigen was added to the blood of ragweed allergic patients in vitro. Franklin and Lowell,[2] using a counting method of greater accuracy, did not observe a significant effect. Black,[3] who observed morphologic changes in the leukocytes rather than changes in their total numbers, concluded that leukocytes do undergo lysis in allergic patients.

In the study of tuberculin hypersensitivity it has been clearly shown that leukocytes undergo lysis in the presence of tuberculin and serum from a sensitized animal.[4, 5, 6] The lytic factor is reported to be thermolabile[7] and the presence of complement is essential.[8]

Leukocytolysis has likewise been demonstrated in immediate types of hypersensitivity, experimentally induced. Using egg albumin and bovine gamma globulin as antigens it has been shown that a thermostable, nonprecipitating antibody is essential.[9, 10] Furthermore, there is evidence to show that the lysis factor is concentrated in Cohn's Fractions I and III of the serum and that it becomes bound to the leukocytes.[11]

Since leukocytolysis has been adequately demonstrated in other hypersensitivity states, we have re-studied this phenomenon in patients with pollen and house dust allergy. The present study includes a larger number of determinations than previously reported by this method. A sufficient number of control observations are included to be statistically significant.

OBJECT OF STUDY

The object of this study is to determine the range of variation in the total leukocyte count of the whole blood of allergic patients when the blood was exposed to pollen and house dust antigens for 80 minutes at 37° C. Normal saline controls were run for each blood. Biological controls consisted of the blood of nonallergic individuals exposed to pollen and house dust antigens for the same time and at the same temperature. An allergic patient was defined as

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one who had a definite history of allergy and whose skin tests were positive to the antigens used in the tests. The time interval of 80 minutes was selected after studies indicated that this interval would detect activity.

METHOD

The method used was a variation of that described by Squire and Lee.[1]

Materials.—Antigens,* pollen 1:20—house dust 1:10; heparinized fresh venous blood—0.1 ml. heparin (1.0 mg.) per 5.0 ml. of blood; and physiological saline.

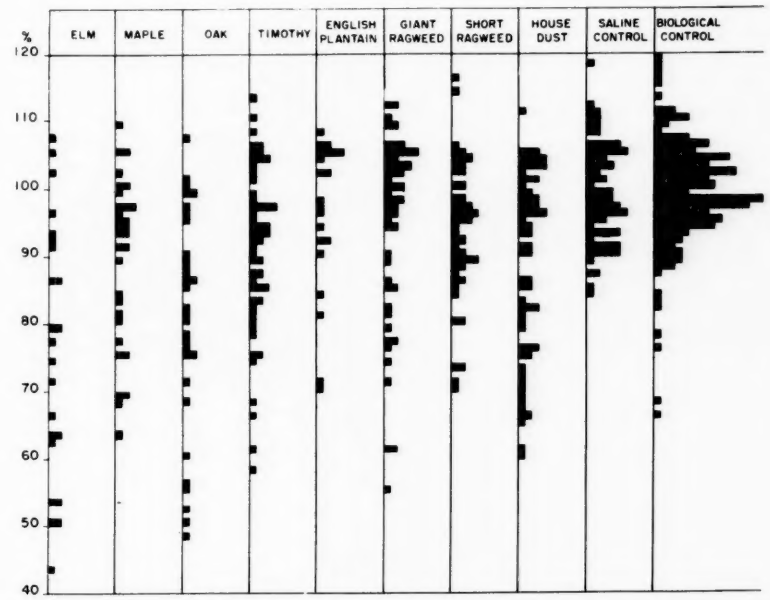

Fig. 1.—Each black square represents the leukocyte count after 80 minutes at 37° C. for each antigen to which the whole blood of the allergic patient was exposed, recorded in percentage of the count at 0 minute. The saline control column shows the result when physiological saline was substituted for the antigen solution. The biological control column gives the percentage count when blood from nonallergic individuals was used in place of blood from allergic patients. The breakdown for the selected antigens composing the totals in the biological control column is given in Fig. 2.

Technique.—(1) Clean 15 by 120 mm. glass tubes were numbered, depending upon the number of antigens to be tested, plus one for the saline control. (2) 0.05 ml. of physiological saline was pipetted into each of the numbered tubes. 0.1 ml. of physiological saline was pipetted into the control tube. (3) 0.05 ml. of 1:20 antigen solution was placed by tuberculin syringe in the appropriate tube. (4) 0.9 ml. of whole heparinized blood, thoroughly mixed, was pipetted into each tube. (5) Tubes were stoppered and mixed. (6) Leukocyte counts were done immediately. (7) Tubes were incubated in the water bath at 37° C. for 80 minutes. (8) After incubation the tubes were again mixed and the leukocyte counts were repeated.

*Obtained from the Hollister-Stier Laboratories in 50 per cent glycerine. Protein nitrogen content: elm, 0.20 mg./ml.; maple, 0.21 mg./ml.; oak, 0.25 mg./ml.; timothy, 0.30 mg./ml.; English plantain, 0.28 mg./ml.; giant ragweed, 0.25 mg./ml.; short ragweed, 0.25 mg./ml.; house dust, 0.27 mg./ml.

Technical Evaluation.—Number of replicates = 10; mean of replicates = 5,860; standard deviation = 364; 2 × SD = 728 = 110 per cent to 90 per cent; expected error of the procedure ± 10 per cent.

RESULTS

The results of these observations are given in Tables I, II, and III, and plotted in Figs. 1 and 2. Reference to Table I and Figs. 1 and 2 shows that of the 328 counts made on the blood of allergic patients, 134, or 41 per cent, fell below 90 per cent; of the 178 biological controls 15, or 8 per cent, fell below 90 per cent; of the 79 saline controls, 5, or 6 per cent, fell below 90 per cent.

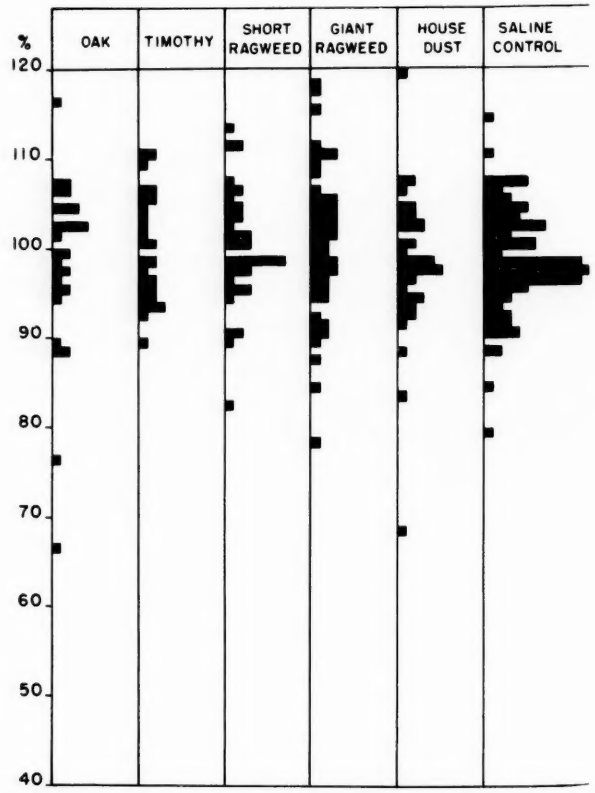

Fig. 2.—Biological Control. Each black square represents the leukocyte count after 80 minutes at 37° C. recorded in percentage of the count at 0 minute for each antigen to which the whole blood of nonallergic individuals was exposed.

The total number of allergic patients was 65; of these 48, or 74 per cent, showed a drop below 90 per cent to one or more antigens. The total number of nonallergic individuals was 178; of these 15, or 8 per cent, fell below 90 per cent.

The order in which drops by antigen occurred in blood of allergic individuals was as follows: elm, 70 per cent, oak, 64 per cent, house dust, 42 per cent, timothy, 41 per cent, maple, 39 per cent, short ragweed, 34 per cent, giant

TABLE I

DATE	PATIENT	ELM	MAPLE	OAK	TIMOTHY	PLANTAIN	GIANT RAGWEED	SHORT RAGWEED	HOUSE DUST	SALINE CONTROLS	BIOLOGICAL CONTROLS
5/ 8/56	C. C.								79	102	
6/20/56	A. F.								85	100	
10/ 6/56	A. F.								93	95	
6/28/56	W. L.								83	111	
7/ 7/56	C. W.								99	90	
9/26/56	A. B.								68	91	
9/28/56	G. V.								102	84	
1/10/58	G. V.				83	84	77	104	81	97	
3/17/59	G. V.				80	71	55	89	73	95	
4/21/59	G. V.	50	69	52	58					99	
10/11/56	J. H.								98	96	
10/11/56	M. S.								98	118	
12/15/56	E. H.		63							93	
12/27/56	S. W.								101	99	
1/22/57	A. O.		97	81			81	86	86	99	
5/28/56	H. S.								101	104	
1/25/57	H. S.			48	66		77	70	90	85	
4/28/57	H. S.			90	93		104	97	105	96	
2/12/57	M. W.				87					101	
2/18/57	P. L.	105	100	75	106	106	96	104	90	105	
3/28/57	D. W.	79	105		78		100	106	60	103	
4/ 9/57	M. H.	107				105	85	89	96	87	
4/29/57	M. H.		97		97	97	105	96	94	108	
4/18/57	J. M.	96		68	99		105	105	96	98	
4/24/57	G. W.				113		106	105	105	104	
4/30/57	H. F.	86	91	56	97	106		89	76	109	
1/ 7/58	H. F.	93	97	88	75	94	104	102	82	105	
1/ 8/58	H. F.				74					106	
5/ 1/57	J. R.	63	95		110		110	71	76	91	
5/15/57	H. M.		69		103	102		103	104	90	
5/28/57	E. L.	66	102	71	105		61	80	72	95	
5/28/57	B. L.			82	92				96	106	
5/29/57	H. K.	50			61		76	73	66	97	
6/ 5/57	D. S.			86					75	98	
6/20/57	N. B.	74	83				74	73	61	89	
6/24/57	T. S.	86	100	100		105	106	80	111	93	
6/27/57	M. S.	79	81	96	82	70	98	92	96	109	
7/ 2/57	R. C.			101	104	81	103		104	99	
7/ 2/57	J. R.	71	96	89	87	98	102	114	67	87	
7/ 5/57	L. C.		75		86		71	95	105	105	
8/28/57	M. N.						79	85		106	
5/22/58	M. N.				101		96	96		97	
9/ 5/57	M. B.	91	80	75	68		85	100	97	110	
10/ 1/57	D. R.			60					75	90	
6/17/58	D. R.			87			98		69	96	
10/25/58	R. T.		105		104		105	98	101	91	
12/ 7/57	F. B.				96		82	102	76	105	
11/ 8/57	N. N.								91	92	
5/27/58	N. N.						105	104	82	93	
11/21/57	S. T.								70	105	
1/ 7/58	M. H.				91		112	95	103	108	
1/21/58	M. S.	102	93		95		112		85	110	
1/22/58	G. H.				106				103	91	
1/24/58	R. W.		75	76	79		103	88	66	96	
5/22/58	R. W.		94	99	94		98	96	86	103	
1/25/58	J. S.		94		94		103	116	103	90	
2/12/58	R. H.	77		86	97	108	94	87	82	106	
3/21/58	I. H.				81		105		99	105	
3/27/58	H. L.		93		90		102	97		101	

(Continued on next page)

TABLE I—CONT'D

DATE	PATIENT	ELM	MAPLE	OAK	TIMOTHY	PLANTAIN	GIANT RAGWEED	SHORT RAGWEED	HOUSE DUST	SALINE CONTROLS	BIOLOGICAL CONTROLS
3/28/58	F. S.				89	105	100	86	80	93	
4/ 7/58	W. D.			85	89	105	61	88	104	97	
4/15/58	J. S.	92		95	108		90	89		111	
4/15/58	N. S.		89	97	104				97	98	
5/ 8/58	M. K.						109	93	100	106	
5/22/58	E. P.				102		86	95		94	
5/28/58	E. M.				92		95	97	97	104	
2/29/58	J. T.	53	84		83	104	106	96	93	96	
6/ 5/58	L. C.				85	96	94	94		102	
6/10/58	M. F.						102		103	103	
6/10/58	H. J.	62	68	78	75		103	98	71	96	
6/11/58	P. R.		95	107	97	100	97	103	94	97	
6/17/58	S. B.				98			90	91	103	
8/12/58	E. E.		91				100	90	65	91	
3/ 2/59	J. A.	43	109	50	105		89			98	
5/15/59	J. A.	63	99	55	94		99		95	93	
4/14/59	E. J.	53	77	95	85	102	101	100	98	101	
4/15/59	W. B.				93	92	105	92		90	
6/ 2/59	T. M.			93	85			91		112	
6/16/59	L. O.			96	93	92	109	84	104	93	
Total	65	23	31	31	53	21	53	50	66	79	178
< 90%	48	16	12	20	22	4	15	17	28	5	15
% < 90%	74	70	39	64	41	19	28	34	42	6	8

TABLE II

	TIMOTHY	SHORT RAGWEED	HOUSE DUST
% < 90% with symptoms	42	36	52
% < 90% without symptoms	28	33	30

TABLE III

	TIMOTHY	SHORT RAGWEED	HOUSE DUST
Scratch Tests			
4+	43	32	53
3+	62	50	35
2+	31	33	29
1+	25	9	100*

*There were two patients with 1+ scratch tests to house dust and both had counts below 90 per cent, giving 100 per cent result.

ragweed, 28 per cent, English plantain, 19 per cent. In the biological control group the order was: oak, 7 per cent, house dust, 5 per cent, short ragweed, 3 per cent, giant ragweed, 2 per cent, timothy, 0 per cent.

Although this experiment was designed primarily as an in vitro test, a clinical correlation was made by reviewing the patients' records and is presented in Table II. The patients were divided into 2 classes: those with clinical symptoms to timothy, short ragweed, and house dust; and those without clinical symptoms to these antigens. It will be seen that there were more patients showing drops with clinical symptoms than without to house dust and timothy but that there was no significant difference in the two groups to short ragweed.

All allergic patients tested had positive skin tests to each antigen used. Correlation of the degree of skin reaction with three of the antigens is shown in Table III. The order of the percentage of patients' bloods showing drops below 90 per cent is 3+, 4+, 2+, 1+, if the 2 patients with 1+ reactions to house dust are disregarded.

SUMMARY AND CONCLUSIONS

The blood of 65 patients was exposed in vitro at 37° C. for 80 minutes to house dust and several pollen antigens. Leukocyte counts were made at the start and the end of the experiment. Normal saline controls and biological controls were run. It was observed that: (1) A significant number of counts fell below the limits of error of the method. (2) There was considerable variation in the leukocytolytic effect among the antigens. (3) Leukocytes of patients with clinical symptoms to house dust and timothy showed greater susceptibility to leukocytolysis than patients with no symptoms to these antigens. This difference was not found in the short ragweed groups. (4) There appeared to be some correlation between the intensity of the skin test and the degree of leukocytolysis.

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A STARCH BLOCK ELECTROPHORESIS STUDY OF AQUEOUS HOUSE DUST EXTRACTS

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IN A PREVIOUS study,[1] a partially purified dust allergen fraction was found to consist predominately of a heterogeneous mixture of acidic polysaccharides with about 5 per cent polypeptide. This fraction contained about equal amounts of uronic acid (probably glucuronic acid), D-glucose, D-galactose, D-mannose, and lesser amounts of L-rhamnose and L-arabinose.

Rimington and co-workers[2] have studied the electrophoretic properties of a purified dust allergen fraction. They found their fraction to consist of two main peaks (phosphate buffer, pH 8, $\mu = 0.2$) of about equal skin reactivity. The material in the fast peak was colored while that in the slow was colorless. By removing the fast and slow materials from the ascending and descending limbs, respectively, of a free boundary cell and analyzing for total hexose and for nitrogen content, it was found that the two materials were chemically similar.

In general it has been found that aqueous dust extracts and their fractions are electrophoretically quite heterogeneous. The starch block electrophoresis method[3, 4] provides a semipreparative procedure for fractionating the dust extracts predominately on the basis of the electrophoretic mobility of the components. With this in mind, we carried out the following experiments to study the relationship between the distribution of components produced by starch block electrophoresis and the distributions of the total carbohydrate, uronic acid, nitrogen, color, monosaccharide composition, and skin reactivity of four dust extracts. Furthermore, by varying the composition of the buffer used for the electrophoresis experiments, some information was obtained regarding the nature of the linkage between the polysaccharide and polypeptide portions of the dust extracts.

Two of the dust extracts studied were prepared in this laboratory and two were commercial preparations commonly used by allergists.

From the Division of Chemistry and Chemical Engineering, The California Institute of Technology, Pasadena, Calif. Contribution No. 2566.

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MATERIALS AND METHODS

Preparation of Dust Extract #110.—A pooled mixture of approximately equal parts of house dust* and mattress dust† was wet with 1.5 m. sodium hydroxide and, after twenty-four hours at 4° C., the liquid was removed with a mechanical press. The pH of the dust extraction mixture was 9.5 two hours after mixing the dust and the sodium hydroxide solution and 8.5 after twenty-four hours when the mixture was pressed. The residue was re-extracted with the sodium hydroxide solution and pressed as before, and the liquid was removed and pooled with the first liquid extract. The pooled solution was filtered through a cotton plug and then centrifuged with a Scharples Super-Centrifuge. The volume was reduced to one seventh of the initial volume by vacuum distillation, keeping the temperature of the dust solution below 40° C. The concentrated dust solution was dialyzed against distilled water and then pervaporated to dryness to recover the solid material.

Preparation of Dust Extract #125.—Mattress dust† was wet with water, frozen in the deep freeze, allowed to thaw, and the fluid expressed by a hydraulic press at 16,000 pounds per square inch. The extract was filtered through glass wool, concentrated to one fourth the initial volume by pervaporation, and centrifuged at about 2,000 g for about forty-five minutes. The supernatant was pervaporated to about one half the initial volume and an equal volume of saturated ammonium sulfate added. The mixture was allowed to stand at room temperature for approximately twelve hours, centrifuged as before, and the supernatant stored at 4° C. As the dust extract was needed, a portion was centrifuged, as above, and then exhaustively dialyzed against distilled water until the dialysate gave a negative test for ammonia with Nessler's reagent. The dust extract was dialyzed against 1 per cent sodium chloride for twenty-four to forty-eight hours and dialyzed against distilled water to remove the sodium chloride. The solution was concentrated for use by pervaporation.

Hollister-Stier Acetone Precipitated Dust Extract.‡—One gram of the dust extract was mixed with 100 ml. of distilled water, allowed to stand at room temperature twenty-four hours, and dialyzed against distilled water for four to five days with two or three changes of dialysis bath per day. The product was centrifuged to remove a small amount of insoluble material. About 30 per cent of the dust extract was found to be soluble and nondialyzable while about 5 per cent was water insoluble. The solution of nondialyzable material was concentrated for used by pervaporation.

Endo House Dust Concentrate.§—The material was supplied as a 2.5 per cent solution of dust extract in 50 per cent aqueous glycerol. The solution

*Collected and donated by various members of the American Academy of Allergy.

†A pool of about 200 pounds of mattress dust from a mattress cleaning establishment in Atlanta, Georgia, was sent to us by Dr. David McK. Pipes.

‡Obtained from Hollister-Stier Laboratories, Inc.

§Obtained from Endo Laboratories, Inc.

was dialyzed against distilled water, as before, and concentrated for use by pervaporation. All the material remained soluble; however, dry weight determinations indicated that about two thirds of the dust extract was dialyzable.

The approximate compositions of the materials are presented in Table I.

TABLE I. APPROXIMATE COMPOSITION OF DUST EXTRACTS

	DUST EXTRACT #110 (%)	DUST EXTRACT #125 (%)	HOLLISTER-STIER EXTRACT (%)	ENDO DUST EXTRACT (%)
Polypeptide	15	10	30	12
Nitrogen	2.5	1.5	4.4	2.0
Polysaccharide	85	90	70	88
Uronic acid	20	25	7.5	10
D-glucose	15	10	20	20
D-galactose	20	30	25	25
D-mannose	15	15	10	15
L-rhamnose	10	10	2	10
fucose	trace	none	none	none
L-arabinose	5	trace	5	5
xylose	trace	trace	none	none
Hexosamine	1.7	0.5	1	2

The polypeptide content is based on nitrogen analysis and the assumption that the polypeptide contained about 16 per cent nitrogen. The nitrogen analyses were carried out by digestion with sulfuric acid and hydrogen peroxide followed by nesslerization.[5] Nitrogen standards and blanks were included and the optical density of the samples, after nesslerization and waiting at least thirty-five minutes, were determined at 440 mμ in the Beckman Model B spectrophotometer. The hexosamine determinations were performed using a modification of the Elson-Morgan procedure.[6, 7] Known amounts (5 to 7 mg.), based on dry weight determinations of solutions of the dust fractions exhaustively dialyzed against distilled water, were hydrolyzed in sealed tubes with 4.0 ml. of 3 N hydrochloric acid for eight hours (at 105° C.).* Dische's carbazole method[8, 9] was used for the uronic acid determinations. The results are reported as per cent equivalent glucuronolactone. In our previous study of a purified dust fraction,[1] we found that the uronic acid content determined by this method was in reasonable agreement with the total number of acidic groups determined by differential titration. The sugar composition indicated in Table I is based on hydrolysis and paper chromatography of the dust extracts. The samples (approximately 3 mg.) were hydrolyzed by heating in sealed tubes with 0.5N sulfuric acid for three hours at 105° C. The paper chromatography methods were the same as those previously described.[1] The relative sugar composition is based on a visual appraisal of the spot intensities in comparison with a standard sugar mixture of a similar sugar composition as the dust extract hydrolysates; it is therefore a very crude estimate. The sugar configurations indicated are based on the polarimeter measurements previously described.[1]

*In our previous paper,[1] it was erroneously reported that the hydrolysis for the hexosamine determination was carried out in 0.5N sulfuric acid. In all cases we have used 3N hydrochloric acid.

Starch Block Electrophoresis.[9]—The apparatus consisted of two cubical Lucite buffer vessels (18 cm. on each edge) and a Lucite tray (1.5 cm. deep, 9 cm. wide, and 44 cm. long) that contained the starch block. Each of the buffer vessels, containing about 3 L. of solution, was divided into four compartments by perforated Lucite partitions. The outermost partitions had platinum wires intertwined through the perforations that were connected to the power supply. The other Lucite partitions were to diminish mixing of the solution in the buffer vessels and thus to keep electrode products away from the starch block. The tray was lined with a sheet of polyethylene and a double thickness of cloth towel placed at each end of the tray before pouring the starch. The ends of the cloth towels provided contact between the starch block and the solution in the buffer vessels. The potato starch was allowed to soak overnight in 1 per cent sodium chloride solution. Before pouring the block, the starch was washed, on a Büchner funnel, twice with 1 per cent sodium chloride and two to four times with the solution to be used as the supporting electrolyte for the electrophoresis run. During the course of washing the starch some of the lighter starch particles were removed by decantation. The washed starch was mixed with the electrophoresis solution to form a suspension of consistency such that it would just flow. The block was poured and the starch grains allowed to settle. The excess solution was removed with blotters. A hole, 1 cm. wide and 7 cm. long, was cut at right angles to the length of the starch block at a position of from 12 to 16 cm., depending on the particular experiment, from the end of the starch block to be attached to the negative terminal of the power supply. Starch was replaced to form a layer of 1 to 2 mm. thickness so the sample did not come in contact with the polyethylene sheet on the bottom of the tray. The sample to be fractionated, in a volume of 5 to 7 ml., was mixed with dry starch and placed in the hole cut in the starch block. After all the sample had been applied to the starch block, the hole was filled to the level of the top of the block with starch wet with the electrophoresis solution. The starch electrophoresis runs were carried out in a coldroom at 4° C. To ensure that the solution levels in each vessel were the same height, a rubber tube connected the two buffer vessels below the solution level. The starch block and the buffer vessels with the electrophoresis solution were allowed to equilibrate in the coldroom at least one hour before clamping the rubber tube connecting the buffer vessels and starting the current. After the current had run the desired length of time, the starch block was removed from the tray and cut into forty-four 1 cm. segments and each portion eluted with the same volume of distilled water. Each eluate was then analyzed and the distribution of various components or properties of the sample on the starch block studied.

Color Determination.—The optical density of each of the eluates was measured at 400 mμ in a Beckman Model B spectrophotometer. A 1 cm. Corex cell was used.

Determination of Total Carbohydrate.[10]—A 0.1 ml. volume of each eluate was mixed with 0.4 ml. of distilled water. Standards containing 10, 20, 30, 40,

50, 70, and 100 γ of galactose per 0.5 ml. and distilled water blanks were included in the determination. The method involves the addition of sulfuric acid and indole and heating in a boiling water bath to develop the color.[10] The optical density of the samples was determined at 470 mμ in a Beckman Model B spectrophotometer. The data were plotted as milligrams of equivalent galactose per milliliter of eluate.

Uronic Acid Determinations.[8, 9]—A 1.0 ml. volume of each eluate was analyzed for uronic acid following the carbazole sulfuric acid method of Dische.[8] The optical densities of each of the eluates, standards (10, 20, 30, 40, 50, 70, and 100 γ of glucuronolactone) and blanks were determined at 535 mμ in the Beckman Model B spectrophotometer. Since a brown color is generated by heating the samples with the acid alone, the determinations were set up in duplicate and the carbazole added to only one series of tubes. The optical densities at 535 mμ determined for the samples with no added carbazole were subtracted from the values obtained for the corresponding samples in the complete test. The data were plotted as γ of equivalent glucuronolactone per milliliter of eluate.

Nitrogen Determination.[11]—Two milliliter volumes of each eluate were evaporated to dryness in a vacuum desiccator over calcium chloride. A micro method, developed by Lanni and his co-workers,[11] was used, with slight modification, to determine the nitrogen content of each tube. A volume of 0.14 ml. of the diluted sulfuric acid, instead of 0.16 ml., was used for the digestion and the tubes were read at 440 mμ in the Beckman Model B spectrophotometer about 35 minutes after the addition of the Nessler's solution. Blanks and standards (10, 20, 30, 40, 50, and 60 γ of nitrogen as ammonium sulfate) were carried through the same analytical procedure. The data were plotted as γ of nitrogen per milliliter of eluate.

EXPERIMENTAL

Starch block experiments, in acetate buffer (pH 4.7, $\mu = 0.1$), were carried out with each of the four dust extracts. Varying amounts of the dust extract, in 5 to 6 ml. of distilled water, were applied to the starch block, 13 to 14 cm. from the end of the block connected to the cathode terminal of the power supply. Samples dialyzed against the acetate buffer gave the same kind of patterns as those in distilled water. There was no insoluble material obtained on dialysis of the dust extracts against the acetate buffer. The following amounts of dust extract, determined by analysis for dry weight, were applied to these starch blocks: extract #110, 154 mg.; extract #125, 180 mg.; Hollister-Stier extract, 68 mg.; and Endo extract, 82 mg. After the period of equilibration in the coldroom (4° C.), a potential of 300 volts (constant voltage output) was applied to the starch blocks for periods of 15.5 to 16.5 hours. Each one-centimeter segment of the block was eluted with 5 ml. and then with 2 ml. of distilled water. The pooled eluates were centrifuged and filtered to remove all the starch grains and the analyses for total carbohydrate, uronic acid, color, and nitrogen carried out on each eluate. The resulting data are

plotted in Figs. 1.1, 2.1, 3.1, and 4.1, along with the data from a blank or control starch block run. In the case of the blank starch block experiment, 5 ml. of distilled water was applied to the block and the current run as usual and all elutions and chemical analyses carried out as in the case of a dust extract sample.

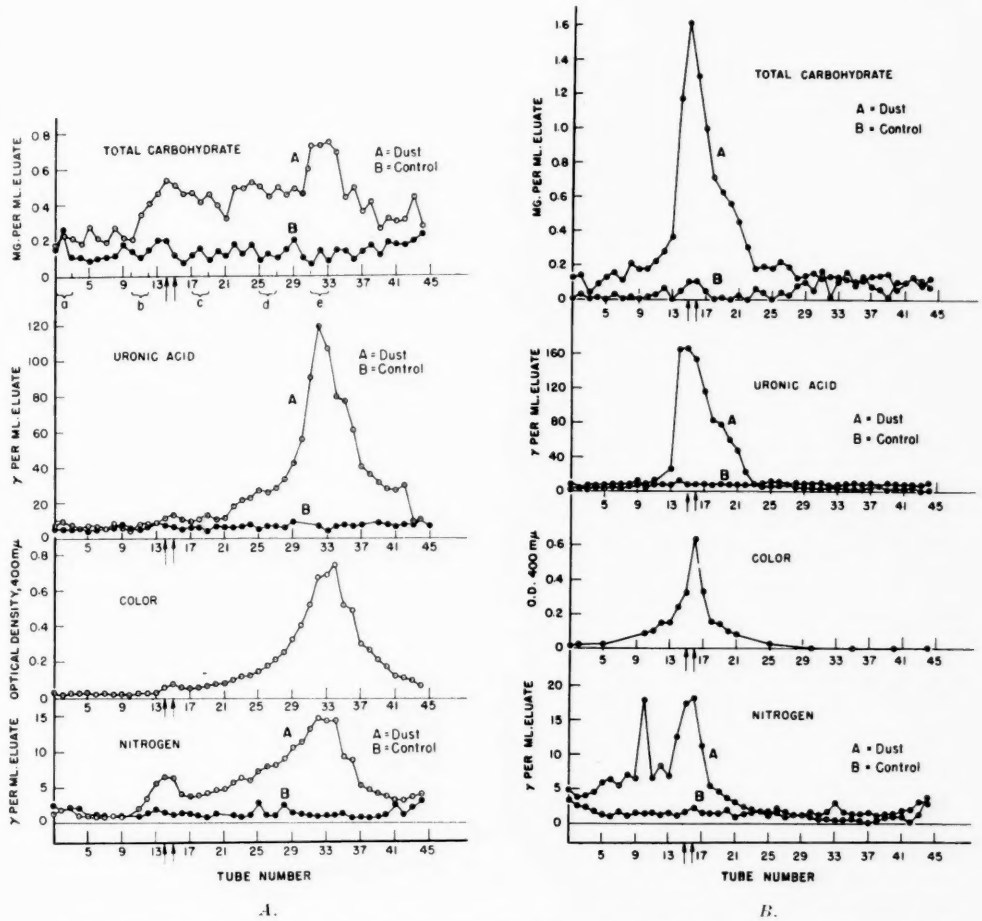


Fig. 1.—Dust extract #110, chemical analyses of starch block eluates. The positive electrode is to the right and the arrows indicate the position of application of the sample. *A*, Acetate buffer, pH 4.7, $\mu = 0.1$. The positions of the letters **a**, **b**, **c**, **d**, and **e** indicate the eluates pooled for chemical analyses and skin testing. *B*, KCl-HCl solution, pH 1.8, $\mu = 0.1$.

Starch block experiments in a KCl-HCl solution (KCl 44.7 Gm., concentrated hydrochloric acid [approximately 12.4N] 16.5 ml., and water to make 8 L. of solution; final pH 1.8, $\mu = 0.1$) were carried out with each of the four dust extracts. Each dust extract, in a volume of 5 to 6 ml., was dialyzed against the KCl-HCl solution and a small amount (approximately 5 to 8 per cent) of insoluble material that formed, at this low pH, was removed by centrifugation. The pH's of the dialyzed dust extracts were found to be within less than 0.1 pH unit of the pH of the KCl-HCl solutions. The sample was

placed 15 to 16 cm. from the cathode end of the starch block, except in the case of the Endo dust sample which was placed at a distance of 24 to 25 cm. The following amounts of dust extract, determined by dry weight analysis, were applied to the starch blocks: extract #110, 165 mg.; extract #125, 94 mg.; Hollister-Stier extract, 68 mg.; and Endo extract, 82 mg. After the equilibration period, a potential of 200 volts (constant voltage output) was applied to

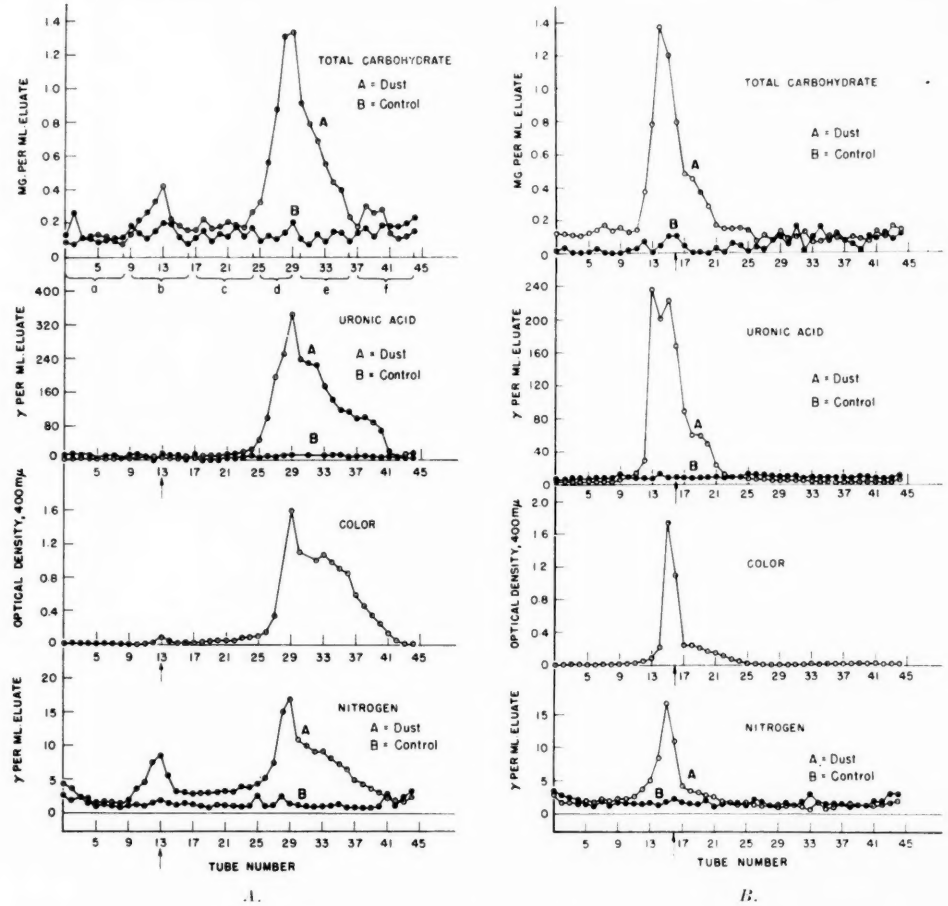
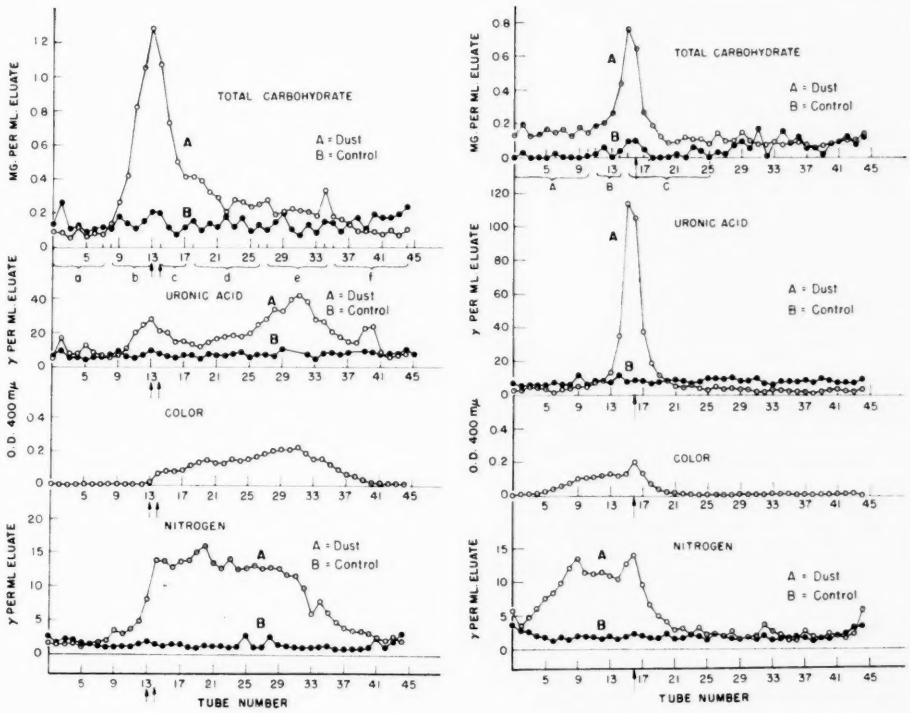


Fig. 2.—Dust extract #125, chemical analyses of starch block eluates. The positive electrode is to the right and the arrows indicate the position of application of the sample. *A*, Acetate buffer, pH 4.7, $\mu = 0.1$. The positions of the letters **a**, **b**, **c**, **d**, **e**, and **f** indicate the eluates pooled for chemical analyses and skin testing. *B*, KCl-HCl solution, pH 1.8, $\mu = 0.1$.

the starch block for 16 to 20 hours. As before, each one-centimeter segment was eluted with 5 ml. and then with 2 ml. of distilled water, the starch grains removed, and each eluate analyzed. The resulting data are plotted in Figs. 1*B*, 2*B*, 3*B*, and 4*B*, along with the data from a blank starch block run. In the case of the blank run, 5 ml. of the KCl-HCl solution, instead of a dust sample, was applied to the starch block and the conditions as well as all analyses were carried out as in the case of a dust sample.

In order to obtain material for hydrolysis and paper chromatography of the polysaccharides in various eluates and for skin testing, additional starch

block fractionations were carried out. In the case of dust extract #110, a starch run in acetate buffer with a 187 mg. sample was carried out for paper chromatography studies and another with a 60 mg. sample for skin testing. In each case the eluates were pooled, on the basis of individual total carbohydrate curves, yielding fractions **a**, **b**, **c**, **d**, and **e** as indicated on the total carbohydrate curve in Fig. 1, *A*. The fractions were dialyzed against distilled water and concentrated by pervaporation. The corresponding eluates from five starch block experiments in the acetate buffer, using varying amounts of the

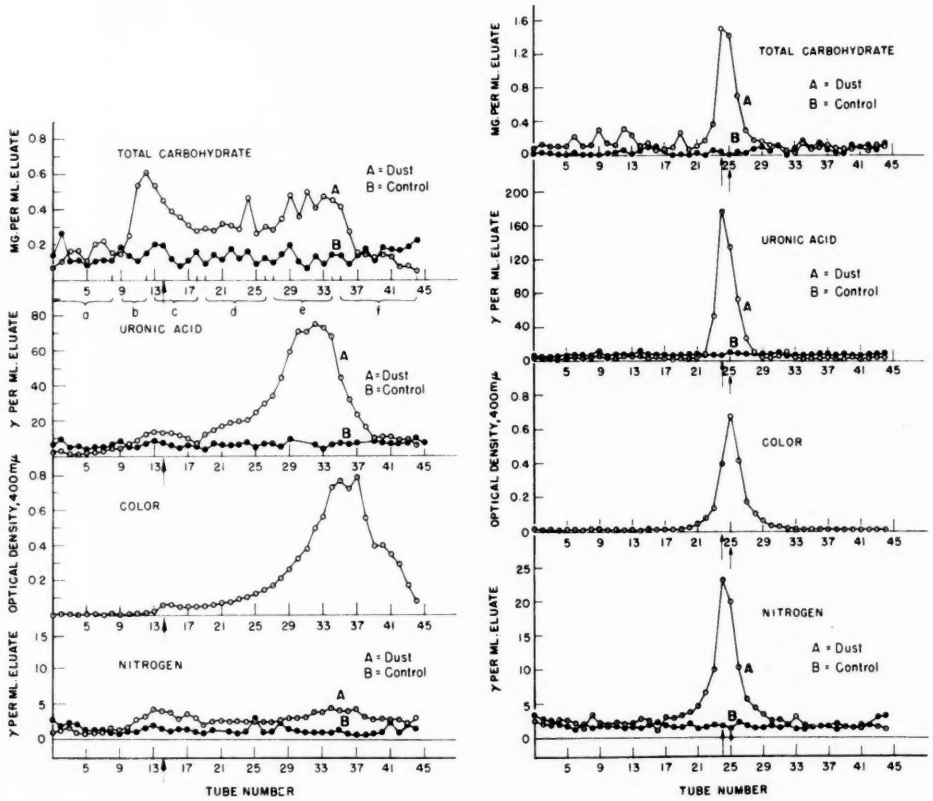


A. *B.*

Fig. 3.—Hollister-Stier extract, chemical analyses of starch block eluates. The positive electrode is on the right and the arrows indicate the position of application of the sample. *A*, Acetate buffer, pH 4.7, $\mu = 0.1$. The positions of the letters **a**, **b**, **c**, **d**, **e**, and **f** indicate the eluates pooled for chemical analyses and skin testing. *B*, KCl-HCl solution, pH 1.8, $\mu = 0.1$. The positions of the letters *A*, *B*, and *C* indicate the eluates pooled for skin testing.

#125 dust extract (82, 82, 141, and 137 mg.) were pooled. Analyses for total carbohydrate were made on each set of eluates and the corresponding samples from each electrophoresis experiment pooled to yield fractions **a**, **b**, **c**, **d**, **e**, and **f** according to the position of the peaks as indicated in Fig. 2, *A*. These fractions were dialyzed and pervaporated, as before, and then skin tested. The relative monosaccharide composition was estimated by hydrolysis and paper chromatography. In the same way, the eluates from corresponding regions of five starch block runs, in acetate buffer, were pooled in the cases of the Hollister-Stier extract and the Endo dust extract. In each case, the corresponding regions of the starch blocks were pooled to form the fractions indicated in Fig. 3, *A* and Fig. 4, *A*. Samples of about 60 mg. were used in the case of the Hollister-Stier

material, while 70 mg. samples of the Endo extract were used. In both cases, the same acetate buffer (pH 4.7, $\mu = 0.1$) was used and a potential of 300 volts applied for about 15 to 16 hours. The one-centimeter segments of the starch blocks were eluted with 5 ml., 2 ml. then with an additional 2 ml. of distilled water in order to increase the yield of material. Samples of about 1 to 2 mg. were hydrolyzed for 3 hours (at 105° C.) in 0.5N sulfuric acid. The samples were neutralized with barium carbonate and analyzed by paper chromatography in two solvents (phenol-water and n-butanol-pyridine-water), in comparison with known sugars, as previously described.[1] The results are presented in Table II. Samples of the various fractions in 50 per cent aqueous glycerol and all



A. *B.*

Fig. 4.—Endo extract, chemical analyses of starch block eluates. The positive electrode is on the right and the arrows indicate the position of application of the sample. *A*, Acetate buffer, pH 4.7, $\mu = 0.1$. The positions of the letters **a**, **b**, **c**, **d**, **e**, and **f** indicate the eluates pooled for chemical analyses and skin testing. *B*, KCl-HCl solution, pH 1.8, $\mu = 0.1$.

at the same concentration with respect to total carbohydrate were tested by direct scratch test in dust-sensitive individuals. In each case, a sample of unfractionated dust extract, at the same concentration as that of its fractions, was tested with the fractions. A dust extract and its fractions were always tested on the same individual at the same time; however, not all the four different dust extracts and their fractions were tested on the same individual at the same time. Therefore, only comparisons among the fractions of the same

TABLE II. ESTIMATION OF THE RELATIVE MONOSACCHARIDE COMPOSITION OF THE STARCH BLOCK FRACTIONS (pH 4.7)*

	GLUCOSE	GALACTOSE	MANNOSE	RHAMNOSE	FUCOSE	ARABINOSE	XYLOSE
#110 fractions							
a	1	0	0	0	0	0	0
b	3	0	1	2	0	trace	0
c	3	0	2	1	0	1	0
d	3	3	2	2	trace	2	0
e	3	2	1	2	1	1	0
#125 fractions							
a	1	0	0	0	0	0	0
b	2	2	2	2	0	1	0
c	2	3	2	1	0	1	trace
d	2	3	1	1	0	1	1
e	2	2	2	2	0	trace	0
f	2	0	1	2	0	0	0
Hollister-Stier fractions							
a	1	0	0	0	0	0	0
b	2	3	2	trace	0	1	0
c	2	3	2	trace	trace	1	0
d	2	3	2	trace	trace	1	trace
e	2	2	trace	1	0	trace	trace
f	2	trace	trace	trace	0	0	0
Endo fractions							
a	1	0	0	0	0	0	0
b	2	2	1	1	0	trace	0
c	2	3	1	2	0	1	0
d	2	3	trace	2	trace	2	trace
e	2	2	0	1	1	1	0
f	2	1	0	1	0	0	0

*The relative amounts of the sugars were graded 0, trace, 1+, 2+, or 3+.

extract are valid. The same patient initials always indicate the same individual. The samples were keyed and randomized before testing to remove any possible bias in reading the skin tests. All the clinical testing was carried out by the direct scratch test method. The skin test data are presented in Tables III, IV, V, and VI. All the patients giving a 1+ reaction or better to any of the samples are included in the tables.

TABLE III. SKIN TEST DATA WITH #110 EXTRACT AND FRACTIONS OBTAINED BY STARCH BLOCK ELECTROPHORESIS (pH 4.7)*

PATIENT	#110	a	b	c	d	e
M. B.†	4		4	3	1	0
P. J.	4		3		3	1
M. B.	4		4		1	1
R. I.†	2		1	3	0	0
R. G.	3		1		1	1
A. G.	1		2		1	1
S. F.	2		1		0	0
P. B.	1		1		0	0
L. B.	2		?		0	?
T. B.	2		0		0	0
T. E.	2		0		0	0
J. C.	0		2		0	0
S. C.	1		0		?	0
R. L.	1		0		0	0
J. V. L.	1		0		0	0

*The concentration of the samples was 0.087 per cent, as equivalent galactose determined by the indole test for total carbohydrate, in 50 per cent aqueous glycerol. The skin reactions were graded 1+ through 4+. The symbol ? indicates a questionable reaction and 0 indicates a negative reaction. A blank space indicates no test was carried out. These were scratch tests.

†These test results were obtained with aqueous solutions of the dust extracts containing no glycerol.

TABLE IV. SKIN TEST DATA WITH #125 EXTRACT AND FRACTIONS OBTAINED BY STARCH BLOCK ELECTROPHORESIS (pH 4.7)*

PATIENT	#125	a	b	c	d	e	f
P. J.	0	0	?	2	3	0	0
C. B.	0	0	2	3	0	0	0
T. B.	2		0	2	0	0	0
G. P.	1		1	0	0	0	1
T. E.	1		0	0	0	0	2
A. G.	1		?	?	1	?	1
P. B.	1		0	1	0	0	1
S. F.	1		0	0	0	0	1
L. B.	?		2	?	0	0	0
J. C.	0	0	0	1	0	0	0
D. D.	?	0	1	0	0	0	0
J. V. L.	1		0	0	0	0	0
S. M.	1		0	0	0	0	0

*The concentration of the samples was 0.2 per cent, as equivalent galactose determined by the indole test for total carbohydrate, in 50 per cent aqueous glycerol. The skin reactions were graded 1+ through 4+. The symbol ? indicates a questionable reaction and 0 indicates a negative reaction. A blank space indicates no test was carried out. These were scratch tests.

TABLE V. SKIN TEST DATA WITH HOLLISTER-STIER EXTRACT AND FRACTIONS OBTAINED BY STARCH BLOCK ELECTROPHORESIS (pH 4.7)*

PATIENT	H. S.	a	b	c	d	e	f
L. B.	4	2	4	4	4	3	?
T. B.	4	2	4	4	3	0	0
R. G.	2	1	3	4	4	2	1
A. G.	3	1	3	4	3	1	2
P. J.	0	1	4	4	4	0	0
C. W.	3	0	4	3	1	0	0
K. N.	2	0	2	3	2	0	0
T. E.	2	0	2	4	0	0	0
S. F.	3	0	2	3	0	0	0
D. D.	1	0	2	4	0	0	0
A. B.	2	0	0	2	1	0	0
C. B.	0	0	2–3	2	0	0	0
J. C.	1	0	1	1	0	0	1
P. B.	0	1	0	1	0	0	1
S. H.	0	0	0	0	1	1	0
R. L.	0	0	0	1	0	0	0
R. A.	0	1	0	0	0	0	0

*The concentration of the samples was 0.2 per cent, as equivalent galactose determined by the indole test for total carbohydrate, in 50 per cent aqueous glycerol. The skin reactions were graded 1 through 4. The symbol ? indicates a questionable reaction and 0 indicates a negative reaction. These were scratch tests.

The curves, shown in Fig. 3, *B*, of the eluate analyses from the Hollister-Stier dust extract run on a starch block with the KCl-HCl solution (pH 1.8, $\mu = 0.1$) showed some relative separation of materials differing in nitrogen contents. In order to determine whether a correlation could be established between the relative nitrogen contents of the materials and the skin reactivity, four such starch block experiments were carried out using varying amounts of the dust extract (65, 65, 65, and 91 mg.). As before the samples were dialyzed against the KCl-HCl solution and a slight residue (5 to 8 per cent) was discarded. The current was applied at a potential of 200 volts for 16 to 17 hours, the starch block cut up in one-centimeter segments and eluted three times with 5 ml., 2 ml., and 2 ml. volumes of distilled water and then the three eluates from each segment combined and analyzed for total carbohydrate by the indole-sulfuric acid procedure. The eluates from the corresponding regions

TABLE VI. SKIN TEST DATA WITH ENDO EXTRACT AND FRACTIONS OBTAINED BY STARCH BLOCK ELECTROPHORESIS (pH 4.7)*

PATIENT	ENDO	a	b	c	d	e	f
A. G.	4	1	1	3	3	1	3
R. G.	2	1	2	3	2	3	2
L. B.	3	0	3	3	3	2	0
T. B.	2	0	3	4	4	0	0
P. J.	2	1	4	1	2	1	1
C. B.	2	0	2	2	2	0	0
C. A. S.	0	0	0	3	3	0	0
J. C.	2	0	1	2	0	0	0
K. N.	0	0	3	0	2	0	0
S. C.	1	0	0	0	3	1	0
P. B.	0	1	0	1	0	1	0
D. D.	?	0	?	1	1	?	0
S. M.	0	0	0	0	1	0	1
C. W.	0	?	0	2	?	?	0
S. F.	0	0	0	0	2	0	0
R. L.	0	0	1	0	0	0	0
G. P.	0	0	0	0	0	0	1
Sylvia M.	0	0	0	1	0	0	0
T. E.	1	0	0	0	0	0	0

*The concentration of the samples was 0.2 per cent, as equivalent galactose determined by the indole test for total carbohydrate, in 50 per cent aqueous glycerol. The skin reactions were graded 1+ through 4+. The symbol ? indicates a questionable reaction and 0 indicates a negative reaction. These were scratch tests.

of the starch blocks were pooled to form fractions A, B, and C as indicated in the total carbohydrate curve of Fig. 3, *B*. These fractions and a sample of the unfractionated Hollister-Stier extract were dialyzed against distilled water, concentrated by pervaporation, diluted to the same concentration, and then made up in 50 per cent glycerol and skin tested as previously described. The data are presented in Table VII.

TABLE VII. SKIN TEST DATA WITH HOLLISTER-STIER EXTRACT AND FRACTIONS OBTAINED BY STARCH BLOCK ELECTROPHORESIS (pH 1.9)*

PATIENT	H. S.	A	B	C
P. J.	2	3	4	3
T. B.	4	4	2	0
R. G.	3	3	2	1
L. B.	3	2	2	0
E. T.	2	2	0	0
A. G.	2	1	1	?
R. A.	1	0	1	0
P. B.	0	0	1	0
J. V. L.	0	1	0	0

*The concentration of the samples was 0.08 per cent, as equivalent galactose determined by the indole test for total carbohydrate (0.15 per cent by dry weight), in 50 per cent aqueous glycerol. The skin reactions were graded 1+ through 4+. The symbol ? indicates a questionable reaction and 0 indicates a negative reaction. These were scratch tests.

Two starch block runs were carried out with the #125 dust extract (180 mg. for the first run and 229 mg. for the second) in a KCl-HCl solution 7M in urea (pH 2.5). The solution was prepared by dissolving 3,360 Gm. of urea (reagent grade), 44.7 Gm. of potassium chloride, and 75 ml. of concentrated hydrochloric acid (12.5M) in water, and diluting to a final volume of 8 L. with distilled water. The samples were placed 15 to 16 cm. from the negative or cathode end of the starch blocks and a potential of 200 volts applied for

forty-eight hours. The color was distributed from the starting position to a distance of about 15 cm. toward the right or anode end of the block. In each experiment the starting position and a segment one centimeter wide on each side was cut out and eluted with 200 to 300 ml. of water on a Büchner funnel. This eluate was designated fraction A. The portion of the starch block from A extending 16 cm. to the right was also cut out and eluted in the same way. This was designated fraction B. The A and B fractions were dialyzed against distilled water for 6 days and the corresponding fractions, A and B, from the two starch block experiments were pooled, pervaporated to a volume of about 10 ml., and dialyzed against 10 L. of distilled water for an additional six days. In each case the dialysis bath was changed two to three times per day. Dry weight and nitrogen determinations showed a nitrogen content of 1.0 per cent for the A fraction and 1.5 per cent for the B fraction. The solutions were analyzed for residual urea by the diacetyl monoxime-arsenic acid method of Rosenthal.[12] The data obtained indicated that less than 5 per cent of the nitrogen in the dust fractions could be accounted for by the possible presence of urea. The solutions were also concentrated, adjusted to the same concentration in 50 per cent aqueous glycerol, and clinically tested. The data are presented in Table VIII.

TABLE VIII. SKIN TEST DATA WITH #125 EXTRACT AND FRACTIONS OBTAINED BY STARCH BLOCK ELECTROPHORESIS AT pH 2.5 IN 7M UREA*

PATIENT	#125	A	B
P. J.	2	2	2
A. G.	1	1	1
T. B.	2	0	0
E. T.	1	1	0
S. M.	1	0	0
J. V. L.	0	0	1
Sylvia M.	1	0	0
R. G.	1	0	?
L. B.	1	0	0

*The concentration of the samples was 0.28 per cent, as equivalent galactose determined by the indole test for total carbohydrate (0.36 per cent by dry weight), in 50 per cent aqueous glycerol. The skin reactions were graded 1+ through 4+. The symbol ? indicates a questionable reaction and 0 indicates a negative reaction. These were scratch tests.

DISCUSSION

The four dust extracts that we have studied by starch block electrophoresis are similar in general chemical composition (Table I) to the purified fraction previously reported.[1] The nitrogen contents of these materials are higher, however, and they are colored, whereas the purified fraction was colorless. Other differences demonstrated are a positive Elson-Morgan color reaction for hexosamine in the case of all four dust extracts and chromatographic evidence for the presence of xylose and fucose in some of the extracts, while the purified fraction appeared to be devoid of these components. Except for the fact that the Hollister-Stier extract has a higher nitrogen content than the other three extracts, the chemical composition of all the extracts is quite similar. Our #110 and #125 extracts have somewhat higher uronic acid contents than the other materials. Possibly this may be due to the fact that

our fractions were derived in part or totally from mattress dust as opposed to house dust. The #110 extract was obtained from half mattress and half house dust while the #125 extract was obtained entirely from mattress dust.

The eluate analyses from the starch block electrophoresis experiments carried out in acetate buffer (pH 4.7, $\mu = 0.1$) show considerable variation in the relative amounts of slow, nearly "isoelectric," materials and rapidly moving, negatively charged, materials. The #125 extract is largely composed of the rapidly moving fraction while the Hollister-Stier extract is largely composed of the slow moving or "isoelectric" fraction and the two other extracts, #110 and Endo, present an intermediate picture. This is shown by a comparison of the total carbohydrate curves in Figs. 1.1, 2.1, 3.1, and 4.1. The uronic acid components are almost exclusively associated with the fast peak in the case of the #110, #125, and Endo extracts, while in contrast the Hollister-Stier extract shows some association of uronic acid containing materials with the slow peak, as shown in the uronic acid curves of Figs. 1.1, 2.1, 3.1, and 4.1. The nitrogen curves in the same figures show that in the case of the #110 and #125 extracts the nitrogen is found to be primarily associated with the fast peak; however, there is some with the slow peak. In the case of the Hollister-Stier and Endo extracts, the nitrogen is found associated with both the fast and slow materials. The nitrogen content of the Hollister-Stier extract is much higher than that of the other extracts and this, in addition to the preponderance of slow material in this extract, possibly explains the much higher association of uronic acid containing components with the slow peak. The polypeptide moiety in the slow fraction of the Hollister-Stier extract possibly provides positive charges to slow down some of the materials in the complex, containing uronic acid, so that they remain in the slow peak. It should be noted that in the case of the Hollister-Stier extract the fast fraction has the higher nitrogen content, while in the case of the Endo extract the fast and slow fractions have more nearly equal nitrogen contents. The distribution of color is shown in the same figures (1.1, 2.1, 3.1, and 4.1). In general, the color is associated with the fast peak. Even in the case of the Hollister-Stier extract, where most of the material is in the slow fraction, the color is predominately associated with the fast moving materials. There is some evidence, especially from the #125 and Endo analyses (Figs. 2, .1 and 4, .1), that the color is moving faster than the bulk of the material in the fast peak. This would suggest that the colored material is not linked through covalent bonds to most of the dust extract molecules. This is in agreement with the fact that we were able to easily separate the colored components from the bulk of the material by basic lead acetate precipitation in the preparation of our purified fraction.[1]

The data from the paper chromatographic study of the relative monosaccharide composition of pooled eluates from the various regions of the starch blocks (as indicated on Figs. 1.1, 2.1, 3.1, and 4.1) are presented in Table II. The general pattern is about the same for all the extracts. Fraction **a**, in all cases, came from a base line region of the starch block and was found to contain only glucose. Fractions **c**, **d**, and **e** generally contain the greatest number of

different monosaccharides, with glucose, galactose, mannose, and rhamnose predominating. It is evident from both Tables I and II that the Hollister-Stier materials are lower in rhamnose than the other extracts. Arabinose was found in all extracts, particularly in fraction **d** which contained materials intermediate in mobility, on the starch block, between the slow and fast peaks and the slow edge of the fast peak. Lesser amounts of fucose and xylose were found especially in the **c**, **d**, and **e** fractions. The fraction **f** containing only the faster moving materials showed a much simpler sugar composition than the factions **c**, **d**, **e**. Except for the #125 fraction, the mannose seems to be concentrated in the slower materials. It should be noted that these results were obtained by hydrolysis of an approximately constant amount of polysaccharide and that when the eluates were obtained from a region of the starch block low in dust polysaccharide the pooled eluates had to be concentrated more and the glucose contribution from the starch became greater. The method employed for hydrolysis and chromatography destroyed uronic acids, therefore the monosaccharide composition presented in Table II is incomplete and should include uronic acids following the distribution indicated in Figs. 1.1, 2.1, 3.1, and 4.1.

The skin test data for the #110 extract and its fractions are presented in Table III. Unfortunately there was not sufficient material of the **c** fraction for complete testing. Most of the activity seems to be confined to **b** and probably **c**, which are the trailing and leading portions of the slow peak. None of the fractions seem to be as active as the unfractionated extract, with the possible exception of **c** where data are lacking. Table IV lists the corresponding data for extract #125. In this case fraction **b**, the slow peak, and fraction **c**, material intermediate between the fast and slow peak, contain most of the activity. The #125 extract and its fractions are all less active than the other materials studied. This may be related to the observation that prolonged contact with ammonium sulfate solution destroys some activity.[13] (The #125 extract was stored in ammonium sulfate at 50 per cent of saturation for many months.) The skin test data for the Hollister-Stier materials are presented in Table V. The most active materials are found, in this case, in both the trailing and leading edge of the slow peak (fractions **b** and **c**). These materials are both apparently a little more reactive than the unfractionated extracts. The intermediate material in fraction **d** seems to be of about the same activity as the unfractionated extract. In the case of the Endo extract and its fractions, most of the activity is confined to fractions **b**, **c**, and **d**, which contain the slow peak and some of the material of intermediate mobility. These three fractions seem to be of about the same activity as the unfractionated extract. It should be pointed out that statements about the relative reactivity of the various fractions are based on the relative intensity of scratch test reactions made at only one concentration. Much more precise ways to measure this would be to carry the reactions to end point, use the passive transfer technique or possibly an allergen neutralization or desensitization test.[14] It is evident from these data that the materials contained in the fast peak, high in uronic acid and containing almost all the color, are much less active than the slow and

intermediate materials. The data also show considerable patient variation with regard to the fraction showing the greatest reactivity. This may be due to the possible presence of a multiplicity of materials of different allergen specificity moving at different rates on the starch block. This is in agreement with our previous observation[1] that some dust-sensitive individuals who gave strong reactions to the less purified extracts did not give any appreciable reaction to the purified fraction.

The problem of trying to correlate the skin reactivity of the fractions with the components as determined by chemical analysis is difficult. In general, it is fairly clear that a high uronic acid content or the presence of color is not necessary for activity. The question arises as to what are the differences evident in our monosaccharide composition data between fractions **d** and **e** of the Hollister-Stier and Endo extracts that could be correlated with the greater skin reactivity of **d** as compared with **e**. There is less mannose and arabinose in **e** than in **d**; however, these differences do not seem great. It is more probable that if the differences in activity are to be correlated with the monosaccharides present, the uronic acid content and the way the monosaccharides are linked to form polysaccharides are the determining factors. It is also possible that the determinant for allergen specificity resides in the polypeptide rather than the polysaccharide portion of the materials. Rimington and colleagues[2] have suggested this on the basis of data correlating the loss of skin reactivity of their allergen fraction, on acid hydrolysis, with the appearance of free amino acids in the hydrolysate.

Dust allergen fractions have been found to be composed of a heterogeneous mixture of acidic polysaccharides with varying amounts of polypeptide components.[1, 2, 15] There are a number of possibilities as to the type of bonding between the polysaccharide and polypeptide components. There may be no linkage between the materials, i.e., the polypeptides and polysaccharides may be present as separate independent molecules in solution. Ionic bonds, such as might exist between the carboxylate ion of a uronic acid residue and a protonated amino group of the polypeptide, may be involved. Covalent bonds such as amide, glycoside, or ester bonds may be present or the two types of materials may be held together by multiple hydrogen bonds, for example, between the carboxylate ion and hydroxyl group of the polysaccharide and the C—O and N—H groups of the amide linkages of the polypeptide.

Starch block electrophoresis experiments were carried out in a strongly acidic solution (KCl-HCl solution, pH 1.8, $\mu = 0.1$) to determine whether the materials were present as separate components in solution or whether they were linked through ionic bonds. In either case, it would be unlikely for the polysaccharide and polypeptide to remain together on the starch block at both pH 4.7 and pH 1.8. At the low pH, the polysaccharide, due to the protonation of its carboxylate ions, should become isoelectric and the polypeptide should become more positively charged, due to protonation of amino groups as well as less negatively charged, due to the protonation of its free carboxylate ions. The data from these experiments are presented in Figs. 1*B*, 2*B*, 3*B*, and 4*B*.

The extract #125 and the Endo extract show no separation of nitrogen from the carbohydrate. The #110 extract and the Hollister-Stier extract show evidence of movement of some of the nitrogen to the left (toward the cathode); however, in both these cases the carbohydrate peaks also extend to the left. The Hollister-Stier extract, more than the other extracts, shows the presence of materials of different nitrogen contents, under these conditions (pH 1.8). Eluates from various regions of the starch block, Fig. 3, *B*, were pooled and skin tested. The data are presented in Table VII. Fractions A and B and the unfractionated extract are of about equal activity while fraction C seems definitely less active. From these data it would appear that the materials of the higher nitrogen content were the most active; however, the starch block electrophoresis fractionation of this extract in acetate buffer (pH 4.7) yielded fractions (**d** and **e**) that were also relatively high in nitrogen content but were less active than fractions of lower nitrogen content (**b** and **c**) as shown in Fig. 3, *A* and Table V. It is possible that small amounts of nitrogeneous materials have rapidly migrated to the left and off the end of the starch block; however, most of the polypeptide components have remained linked to the polysaccharides under these conditions. Therefore it is unlikely that the polypeptides are primarily present as separate components or are linked to the polysaccharides through ionic bonds.

In order to test the hypothesis that the polysaccharide and polypeptide moieties were linked by multiple hydrogen bonds, a starch block electrophoresis experiment was carried out in 7M urea at pH 2.5. The use of concentrated urea solution (6 to 7M) is the classical way to separate into subunits macromolecules that are held together by multiple hydrogen bonding. Concomitant electrophoresis at pH 2.5 should serve to separate any dissociated polypeptide from the polysaccharide. Fractions A and B obtained by the starch block electrophoresis of samples of #125 extract under these conditions had nitrogen contents of 1.0 and 1.5 per cent respectively. The unfractionated dust extract had a nitrogen content of 1.5 per cent. These data indicate that no separation of the two moieties was obtained and that the polysaccharide and polypeptide are probably not linked by multiple hydrogen bonds.

As a result of these experiments, it is evident that the polysaccharide-polypeptide bonding is primarily covalent.

The control experiments carried out with both the acetate buffer and the HCl-KCl solution show that some soluble carbohydrate as well as a little soluble nitrogenous material were extracted from the starch along with the dust extract fraction. In most cases this does not confuse the interpretation of the curves since the patterns obtained with the dust eluates were either fairly high or down close to base line. No peak should be considered significant, if it is based on one point alone. As an example, the high nitrogen content found in tube number 10 of Fig. 1, *B* probably has no significance.

The final pattern obtained by starch block electrophoresis is the end result of a number of processes. By far the most important of these is electrophoretic transport of the materials; however, electroosmosis, adsorption, and ion exchange also play some part.

SUMMARY

1. The approximate chemical compositions of four dust extracts, including the Hollister-Stier and Endo commercial preparations and two extracts prepared in our laboratory, were determined. These materials were found to consist of a heterogeneous mixture of acidic polysaccharides with varying amounts of polypeptide.

2. The materials were investigated by starch block electrophoresis in acetate buffer (pH 4.7, $\mu = 0.1$). In general, a fast (negatively charged) and a slow peak were found. The allergen activity was predominately associated with the materials of slow or intermediate mobility. In some cases the fractions seemed to be more active than the unfractionated extract.

3. No simple relation was evident between the monosaccharide composition or the nitrogen content of the fractions and the allergen activity.

4. The color moved slightly faster than the fast peak and was definitely separated from the more active fractions.

5. Experiments were presented that indicated that the polypeptide moiety of the dust extract was linked to the polysaccharides by covalent bonds.

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FIBRINOLYSIS AND ANAPHYLAXIS

I. Activation of Blood Fibrinolysin During Acute Anaphylactic Shock in Guinea Pigs

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IT HAS LONG been suspected that activated blood fibrinolysin may play a prominent role in the pathogenesis of anaphylactic shock. In human beings the blood exhibits an abnormally high "fibrinolytic activity" in a number of pathologic conditions, and even in various physiologic states, especially those involving a degree of stress.[1] Most interesting is the recent observation of Lowell, Franklin, Schiller, and Follensby[2] that blood collected from patients undergoing induced mild systemic allergic shock may show an abnormally increased *thrombolytic activity*, reflected in the rapid, spontaneous lysis of clotted blood samples. (Sherry and associates[1] recommended that the process by which a blood clot is lysed through activation of its contained plasminogen be called *thrombolysis* rather than *fibrinolysis*, which describes only part of a complicated process.) The demonstration by Rocha e Silva, Jaques, and associates in 1946-1947 that specimens of clotted whole blood, collected during anaphylactic or peptone shock in dogs, are rapidly re-dissolved spontaneously has been interpreted as strong support for the view that activation of blood fibrinolysin is a *primary* event in anaphylaxis, initiating histamine release and the subsequent shock reaction.[3, 4, 5]

Recently, however, the situation has been recognized to be much more complex.[6, 7] Unequivocal proof that plasminogen activation is a prime factor in allergic reactions has not been forthcoming, and the probable participation of enzymes outside the plasminogen-plasmin system, especially activated complement (C'1 esterase),[8] or other proteases of unknown origin[9, 10] has been suggested.

Nevertheless it seemed desirable to extend observations on spontaneous clot lysis during experimental anaphylactic and anaphylactoid shock to species other than the dog, and especially appropriate to make systematic studies of this kind with guinea pigs. To understand allergic mechanisms it is clear that each species needs to be studied for itself.[11]

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The present report describes the results of whole blood clot lysis tests made during acute anaphylactic reactions in guinea pigs, as well as certain control experiments on the comparative effects of simple anoxia on clot lysis, and on the mechanism by which protamine, added to the test specimens, enhances thrombolysis.

METHODS AND MATERIALS

Sensitization of the Guinea Pigs.—Guinea pigs of either sex, weighing approximately 300 to 400 grams, were passively sensitized by the intraperitoneal injection of an amount of rabbit anti-crystalline egg albumin serum (anti-CEA) containing 0.1 to 0.2 mg. antibody nitrogen.

Collection of Blood Specimens.—The sensitized animals were challenged after 36 to 72 hours by the intracardial injection of CEA antigen. For bleeding and intracardial injection, the guinea pigs were tied down on their backs in a specially designed cradle; no anesthesia was used. After clipping the hair over the thorax, and wiping with alcohol, a first blood sample was drawn into a 2 ml. syringe, previously rinsed with saline, by introducing the needle directly into

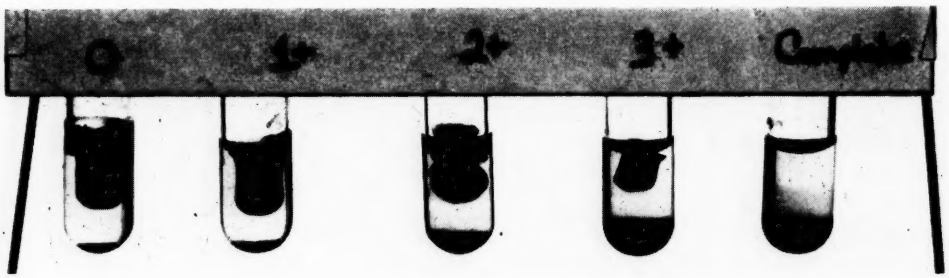

Fig. 1.

the heart through the chest wall. After obtaining this before-injection blood sample, the syringe was detached, leaving the needle in the heart. A second syringe containing the antigen (or control reagent) was then connected to the needle, and the material was injected. In about 30 seconds, at the beginning of anaphylactic shock, this syringe was now replaced by a third saline-rinsed syringe into which was drawn at once the second sample of blood. Both syringe and needle were then withdrawn. The third blood sample was taken by puncturing the heart again at the height of anaphylactic symptoms, using still another syringe. Finally, when the characteristic gasping indicated that death was imminent, the chest was quickly opened and a death specimen was obtained with a fifth syringe directly from the still-beating heart.

Clot Lysis Tests.—A 0.25 ml. portion of each freshly drawn blood sample was introduced directly into a previously prepared test tube containing 0.25 ml. sterile veronal buffer, pH 7.5. Another 0.25 ml. portion was added to a parallel tube containing 0.25 ml. sterile solution in buffer of protamine sulfate (salmine)* having 200 μg of protamine per milliliter. Advantage was thus taken of the

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fact that salmine, in appropriate concentrations, enhances fibrinolysis.[4, 5, 12, 13] Duplicate sets of both the buffer and the protamine tubes were set up in most experiments. As soon as all specimens from an animal had clotted, that is, within two minutes, the tubes were placed in a 37° C. water bath and the time noted. Readings of the degree of clot lysis were made by simple inspection at intervals, and recorded at one, two, four, and twenty-four hours of incubation. Complete lysis of the clot was evident when only a deposit of free red blood cells remained in the tube (Fig. 1).

SUMMARY OF OBSERVATIONS

Clot Lysis in Blood Specimens From Normal and From Passively Sensitized Guinea Pigs Before any Intracardial Injection.—A certain amount of spontaneous lysis occurred within twenty-four hours among clotted blood specimens obtained from normal guinea pigs never injected, and from animals which had received, 48-72 hours before, an intraperitoneal injection of rabbit antiserum. The data from ninety-two guinea pigs are summarized in Table I. It will be noted that, in the blood samples incubated in buffer, the thrombolytic activity was generally low, and nearly equal in the sensitized and in the normal animals.

TABLE I. CLOT LYSIS IN NORMAL AND SENSITIZED GUINEA PIGS

WHOLE BLOOD SPECIMENS FROM	NO.	NO CLOT LYSIS	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
			+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
SPECIMENS INCUBATED IN BUFFER SOLUTION									
Normal guinea pig (never inoculated)	46	34	12						
Sensitized guinea pig (rabbit anti-serum, i.p.)	46	39	4	3					
SPECIMENS INCUBATED IN PROTAMINE SOLUTION									
Normal guinea pig (never inoculated)	46	20	20	5	1				
Sensitized guinea pig (rabbit anti-serum, i.p.)	46	13	13	11	8	1			

The portions of the same bloods incubated in protamine solution showed the expected increase in susceptibility to spontaneous lysis. Possible mechanisms are discussed below. The samples from the passively sensitized guinea pigs showed somewhat more thrombolysis than those from normal animals. No ready explanation for this observation is available. All these findings support the belief that the fibrinolytic system is normally in a state of dynamic activity.

Clot Lysis in Blood Samples Collected During Acute, Fatal Anaphylactic Shock.—In Tables II and III are summarized the results of thrombolysis tests with samples from twenty-seven passively sensitized guinea pigs, all of which died in typical anaphylactic shock within four to eight minutes after intracardial injection of the antigen. In the buffer-incubated specimens (Table II), there is a consistent increase in the extent and speed of the clot lysis in successive samples taken during the course of the shock reaction. At the height of

the shock, complete lysis occurred in four samples (25 per cent) in twenty-four hours, and about 18 per cent of the death specimens were lysed completely, two of them within two hours.

TABLE II. CLOT LYSIS IN ACUTE, FATAL ANAPHYLACTIC SHOCK

SPECIMENS INCUBATED IN BUFFER SOLUTION									
WHOLE BLOOD SPECIMENS COLLECTED	NO.	NO CLOT LYSIS	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
			+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Before shock injection	26	21	3	2					
1 Min. after shock injection	26	20	2	1	1	2			
At height of shock	16	7	4	1		1	3		
At death	27	11	3	3	5	2	1	2	

TABLE III. CLOT LYSIS IN ACUTE, FATAL ANAPHYLACTIC SHOCK

SPECIMENS INCUBATED IN PROTAMINE SOLUTION									
WHOLE BLOOD SPECIMENS COLLECTED	NO.	NO CLOT LYSIS	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
			+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Before shock injection	27	8	7	8	3	1			
1 Min. after shock injection	26	6	4	4	5	3	3	1	
At height of shock	16		1	3	5	2	4	1	
At death	27	1		3	4	4	11	2	2

The same phenomenon is shown in much stronger degree by those portions of the same blood samples that were incubated in protamine solution, instead of plain buffer. Activation of the thrombolytic process began within the first few seconds of shock, and the clot lysis became increasingly active until 70 per cent of the death specimens were completely lysed, most of them within four hours or less.

Clot Lysis in Blood Specimens Collected From Sensitized Guinea Pigs Having Mild or Subclinical Reactions to Antigen.—A number of sensitized guinea pigs, when challenged with antigen intracardially, developed only mild and transient anaphylactic symptoms or no definite signs of illness. Some received an inadequate sensitizing dose of antiserum or too small a dose of antigen to produce fatal shock. Others were deliberately made partially antianaphylactic by previous small injections of antigen. The results of clot lysis tests with thirty-four blood samples taken immediately before and about one minute after an intracardial antigen injection in animals with reactions of this kind are presented in Table IV.

It will be seen that the degree of thrombolysis was not significantly higher in the second than in the first specimens among those incubated in buffer solution, and the second samples were only about 10 per cent higher among those tested in protamine solution.

These findings, in comparison with those obtained with specimens from animals dying in acute shock, would seem to indicate that the increased thrombolysis during anaphylaxis is roughly proportional to the severity of the reaction. It is evidently not the result of the mere combination of antigen and antibody in vivo, unless this leads to the appearance of frank anaphylactic symptoms.

TABLE IV. CLOT LYSIS IN SENSITIZED GUINEA PIGS HAVING MILD ANAPHYLACTIC SHOCK OR NO SYMPTOMS FOLLOWING INTRACARDIAL INJECTION OF ANTIGEN

WHOLE BLOOD SPECIMENS INCUBATED IN	NO.	NO CLOT LYSIS	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
			+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Buffer									
Before shock injection	34	26	6	1	1				
1 min. after injection	32	27	3			1			1
Protamine									
Before shock injection	33	13	13	4	3				
1 min. after injection	32	15	7	5	2	1	1		1

Clot Lysis in Blood Samples Collected During Induced Anoxia in Normal Guinea Pigs.—Early in these studies with animals in anaphylactic shock, it occurred to us that the changes in the blood could be due, in part, to the sudden anoxia developing in the guinea pigs during the anaphylactic process. To test this possibility, a group of thirteen normal guinea pigs was sacrificed by producing rapid death through strangulation or through suffocation induced by fastening a plastic bag over the head. Blood specimens for clot lysis tests were collected as usual, including a sample from each of ten additional normal animals of the same lot.

The results of the tests with the specimens in protamine solution are summarized in Table V. None of the bloods from the healthy guinea pigs before their sacrifice showed complete lysis, and six (26 per cent) did not lyse at all.

TABLE V. CLOT LYSIS IN GUINEA PIGS: ANOXIC DEATH

SPECIMENS INCUBATED IN PROTAMINE SOLUTION									
WHOLE BLOOD SPECIMENS COLLECTED	NO.	NO CLOT LYSIS	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
			+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Before anoxia	23	6	4	7	6				
Distress symptoms (1-2 min.)	12	1		2	4	3	2		
Height of distress	7	1		1	2	1		2	
At death (7-10 min.)	13				5	2	5	1	

As an anoxic condition was induced, however, the blood clots showed increasing thrombolytic activity. At death, all thirteen samples had an advanced degree of lysis, and more than half of them (61.5 per cent) were completely lysed within twenty-four hours. The corresponding figure for the specimens from the twenty-seven guinea pigs that died of anaphylactic shock (Table III) was 70.3 per cent. Thus, anoxia alone was accompanied by almost the same degree of spontaneous clot lysis as death from acute anaphylaxis.

Observations on the Mechanism of the Protamine Effect In Vitro.—We wish to emphasize that the preceding findings on the increase in spontaneous thrombolysis in clotted blood samples obtained from guinea pigs during anaphylactic shock and anoxia were clearly shown in samples of whole blood, diluted 1:1 in veronal buffer only, and that the parallel sets of tubes containing protamine

merely revealed the same relative increases in an accentuated degree. The precise mechanism by which protamine brings about this enhancement of clot lysis has not been fully elucidated. Jaques and Waters[12] and Rocha e Silva and associates[4, 5] added protamine to blood samples from dogs in anaphylactic and peptone shock to neutralize the heparin present (which might inhibit fibrinolysis), so that they could then better detect and measure fibrinolytic activity in the specimens. They observed, however, that protamine enhanced fibrinolysis in vitro, even in the absence of heparin, and antagonized the natural protease inhibitor in untreated dog serum.[5] This suggested to us the use of this reagent in the present work.

Von Kaulla[13] has presented further evidence that protamine inactivates an inhibitor of fibrinolysin. This we have confirmed by use of the casein digestion techniques developed in this laboratory for titrating the proteolytic activity of serum.[14, 15] We have found that pre-mixture of human or guinea pig serum with protamine (salmine) in the final concentration of 200 μg per milliliter of blood, as in the clot lysis tests under discussion, removes or inactivates an inhibitor of plasminogen activation. The total proteolytic titer of such pretreated serum (after removal of the protamine precipitate by centrifugation) is always definitely higher than that of the untreated serum. Our tests also established the significant fact that the addition of even twice the amount of protamine routinely used in the in vitro clot lysis tests does not in itself cause the appearance of any proteolytic activity.

The effect of the amount of salmine used in clot lysis studies was investigated by running, in parallel, along with plain buffer tubes, protamine tubes containing final concentrations of 20 μg, 100 μg, 200 μg, and 400 μg per milliliter of blood, respectively. It was found that 400 μg per milliliter prevented the formation of a complete clot, and these tubes gave no useful information on incubation. In the 200 μg protamine per milliliter tubes clotting was always slightly delayed by about two minutes, but the clots, once formed, were firm and complete, and outwardly indistinguishable from those in the plain buffer-blood tubes. With the smaller concentrations of protamine (20 μg and 100 μg per milliliter), the delay in clotting was less noticeable. In the case of ninety-five specimens from thirty-four different guinea pigs, tested in parallel, essentially the same relative changes in the degree of spontaneous clot lysis in specimens collected during anaphylactic shock were observed in all these three concentrations of protamine. For subsequent routine clot lysis tests, a concentration of 200 μg per milliliter was chosen, since it gave the greatest amount of thrombolysis, without apparently interfering with the clotting of the original blood sample, except to delay it somewhat. It would seem possible, however, that some alteration in the intimate structure of the clot occurs when it is formed in the presence of protamine, which favors spontaneous lysis when the contained plasminogen becomes activated.

DISCUSSION

The results of this investigation would seem to indicate that the use of whole fresh blood, diluted 1:1 in veronal buffer at pH 7.5 (with or without

added protamine), supplies suitable specimens for observing the spontaneous lysis of guinea pig blood clots; any labile fibrinolytic elements are presumably stabilized in the clot as in the case of human bloods.[16] When the blood samples clot in the presence of a suitable amount of protamine, the subsequent thrombolysis is of the same order as in the plain buffer specimens, but of greater rapidity and extent, thus providing a more sensitive test, without introducing any recognizable source of error.

Sherry and associates have found that with human bloods excessive spontaneous clot lysis is accompanied by an abnormal increase of plasminogen *activator* in the circulating blood. Since both activator and plasminogen are strongly absorbed onto fibrin during the clotting process, the excess of the former favors prompt activation of the plasminogen within the clot, with the resulting rapid thrombolysis.

In guinea pig serum, however, an activator principle comparable to that in human serum is normally either absent, or present in extremely small amounts.[17] It has been suggested that the guinea pig fibrinolytic system is simpler than that in human blood, consisting principally of plasmin in a complex with antiplasmin which is readily dissociated.[18] However, the recent studies of Olesen[19] have shown that production of fibrinolytic activity in euglobulin preparations from guinea pig serum by addition of peptone is mediated through a precursor system, functioning in a manner similar to the "pro-activator system" in human serum which is acted upon by streptokinase. It must be stated that at present the mechanism by which clotted guinea pig blood acquires the capacity for abnormal spontaneous lysis has not been established, but it probably involves, as in the case of human bloods, an increase in an activating substance.

SUMMARY AND CONCLUSIONS

With the techniques employed in this study, a certain minimal amount of spontaneous dissolution of clotted blood samples from normal guinea pigs, never inoculated, was observed. Also, specimens from guinea pigs that had been given only a single intraperitoneal injection of sensitizing rabbit antiserum showed a somewhat greater amount of clot lysis. This thrombolytic tendency was markedly increased when the animals were thrown into acute anaphylactic shock, and the degree and speed of the clot lysis was progressively greater in successive specimens taken from the beginning of the reactions to death. The process apparently began almost immediately on injection of the specific antigen, since specimens collected within seconds already showed an increase in thrombolytic activity.

This apparent close association of the accelerating thrombolysis with the development of the acute symptoms of anaphylactic shock does not, in itself, prove that fibrinolysin activation is the *primary* cause of the anaphylactic reactions, as originally held by Rocha e Silva and others. Our control studies would suggest that early anoxia may be responsible for some of the abnormally active clot lysis observed during severe anaphylactic reactions.

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The reported observations permit only the conclusion that abnormal activation of fibrinolytic enzymes in clotted blood samples is a regular feature of severe or fatal anaphylactic shock in guinea pigs. The true significance of this finding in relation to the basic mechanism of anaphylaxis must await further study. Investigations of clot lysis in anaphylactoid shock and in other control experiments are indicated, together with analysis of changes in components of the plasminogen-plasmin system in the serum of the experimental animals. Studies of this kind are in progress.

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FIBRINOLYSIS AND ANAPHYLAXIS

II. Activation of Blood Fibrinolysin During Acute Anaphylactoid Shock in Guinea Pigs

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IN A PREVIOUS communication,[1] we have described the abnormal thrombolytic activity which parallels the acute anaphylactic shock reaction in sensitized guinea pigs. Specimens of whole clotted blood collected during the shock and incubated at 37° C., showed an abnormal capacity for rapid spontaneous lysis of the clots. This present report is an extension of that study to determine the degree of clot lysis in guinea pigs which accompanies acute anaphylactoid shock. For this purpose, groups of normal guinea pigs were subjected to injections of peptone, histamine, trypsin, and rat anaphylatoxin.

MATERIALS AND METHODS

The animals used were normal guinea pigs, weighing 300 to 450 grams, that had never been previously inoculated. Specimens of whole blood were collected as previously described by cardiac puncture, without anesthesia, before and just after the intracardial injection of the shocking material (or control reagent), at the height of the shock reaction, and at death. The blood samples were placed directly into tubes containing an equal volume (0.25 ml.) of sterile veronal buffer, pH 7.5, and into other tubes containing a solution of protamine sulfate (salmine), in veronal buffer, having 200 μg of protamine per milliliter. Duplicate sets of both the plain buffer and the protamine buffer tubes were set up in most experiments.

As soon as the blood specimens had clotted the tubes were placed in a 37° C. water bath and the time noted. The tubes were closely observed for evidences of spontaneous lysis of the clots, and a record made at intervals of one, two, four, and twenty-four hours of incubation. Three degrees of partial clot lysis (+, ++, and +++) were recognized by comparison with photographs showing the typical appearance of these stages of lysis. Complete lysis was evident when the sample became entirely fluid and contained only a deposit of free red blood cells.[1]

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EXPERIMENTAL RESULTS

Clot Lysis in Fatal, Acute Peptone Shock.—Witte's peptone was dissolved in saline in the amount of 200 mg. per milliliter and the solution was sterilized by autoclaving. Eleven normal guinea pigs were injected intracardially with 1 ml. of this solution. The resulting acute reactions closely resembled those seen in sensitized animals injected with specific antigen. Death ensued in three to ten minutes, and an immediate autopsy showed the typically inflated lungs, and still-beating heart characteristic of both anaphylactic shock and of this kind of anaphylactoid shock.

The results of the clot lysis tests are presented in Table I. It will be seen that an abnormal thrombolytic activity appeared in the clotted blood samples obtained within one minute after the peptone injection. As in the case of bloods

TABLE I. CLOT LYSIS IN PEPTONE SHOCK

WHOLE BLOOD SPECIMENS	NO. OF GUINEA	NO CLOT	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS	
TIME OF COLLECTION	PIGS	LYSIS	+	++	+++	24 HR.	4 HR.
Incubated in Plain Buffer							
Before shock injection	11	9	2				
1 min. after shock injection	11	4	2	2	1	2	
At height of shock	9	1		2		3	3
At death	10	1	1	3		4	1
Incubated in Protamine Buffer							
Before shock injection	11	3	6	1	1		
1 min. after shock injection	11	3	2	1	1	4	
At height of shock	9	1			1	2	5
At death	11	2	1	1	2	4	1

from guinea pigs during specific anaphylactic shock,[1] the extent and speed of clot lysis increased still further in the later specimens, especially those collected at the height of the shock. In the latter, complete lysis occurred in 67 per cent of the samples incubated in plain buffer and in 78 per cent of those incubated in protamine-buffer solution.

Clot Lysis in Histamine Shock.—Twenty-three normal guinea pigs were injected intracardially with doses of histamine diphosphate or histamine acid phosphate in sterile saline containing 0.3 to 0.5 mg. per milliliter. The animals went into a rapidly fatal acute shock, showing at autopsy the markedly inflated lungs characteristic of histamine death.

Nearly all the blood specimens collected immediately after the histamine was injected, as well as those obtained at the height of the reaction and at death, were lysed spontaneously in some degree within twenty-four hours (Table II). Thirteen (65 per cent) of the one-minute-after-injection samples incubated in plain buffer and seventeen (77 per cent) of these same specimens incubated in protamine buffer were lysed completely, the majority after only four hours of incubation time.

Clot Lysis in Trypsin Shock.—An intracardial injection of a solution in saline containing 0.5 mg. of crystalline trypsin was used to produce acute trypsin

TABLE II. CLOT LYSIS IN HISTAMINE SHOCK

WHOLE BLOOD SPECIMENS	NO. OF GUINEA	NO CLOT	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
TIME OF COLLECTION	PIGS	LYSIS	+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Incubated in Plain Buffer									
Before shock injection	21	16	5						
1 min. after shock injection	20	1	2	2	2	3	6	1	3
At height of shock	20	2	0	4	5	3	4	1	1
At death	22	1	1	8	3	1	2	2	4
Incubated in Protamine Buffer									
Before shock injection	23	10	12	1					
1 min. after shock injection	22	1	0	2	2	2	8	3	4
At height of shock	20	0	1	2	4	5	4	1	3
At death	23	1	2	1	2	3	7	2	5

shock, with death in five to nine minutes. The signs of respiratory distress were not as prominent in these animals as in the guinea pigs shocked with histamine or peptone, and the lungs at autopsy were only slightly inflated, or apparently normal.

The effect on clot lysis of the trypsin injection in nine normal guinea pigs is presented in Table III. A remarkable acceleration of thrombolysis occurred, resulting in complete lysis within one or two hours in all specimens collected after the shock and incubated in protamine buffer. Likewise the majority of the blood samples incubated in plain buffer were completely lysed within two hours, and the remainder within four hours.

TABLE III. CLOT LYSIS IN TRYPSIN SHOCK

WHOLE BLOOD SPECIMENS	NO. OF GUINEA	NO CLOT	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
TIME OF COLLECTION	PIGS	LYSIS	+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Incubated in Plain Buffer									
Before shock injection	9	5	3	1					
1 min. after shock injection	9							3	6
At height of shock	8							3	5
At death	8							2	6
Incubated in Protamine Buffer									
Before shock injection	9	4	4	1					
1 min. after shock injection	9							3	6
At height of shock	8						1	2	5
At death	9						2	3	4

Clot Lysis in Anaphylatoxin Shock.—Anaphylatoxin was prepared from rat serum by the agar method of Bordet,[2] as described by Rothschild and Rocha e Silva.[3] Portions of fresh rat serum (usually 10 ml.) warmed to 37° C., were thoroughly mixed aseptically with an equal volume of 0.25 per cent solution of agar in saline, also at 37° C. The agar-serum mixture was incubated at 37° C. for fifteen to thirty minutes, then centrifuged. The clear supernatant over the sedimented agar was collected as the anaphylatoxin.

Lots of rat anaphylatoxin thus prepared were injected intracardially into eight normal guinea pigs in an amount equal to 1 per cent of the body weight.

The animals developed acute illness, with the symptoms characteristic of specific anaphylactic shock and of histamine shock. Death occurred in an average time of about five minutes, and autopsies revealed the typical gross pathological changes (inflated lungs), while the heart continued to beat for several minutes.

A pattern of increasing clot lysis during the reaction, similar to that noted above with peptone and histamine shock, was exhibited in these animals (Table IV). In the death specimens, complete lysis occurred within twenty-four hours in five out of seven of the bloods incubated in plain buffer, and in six out of eight of the samples incubated in protamine-buffer.

TABLE IV. CLOT LYSIS IN ANAPHYLATOXIN SHOCK

WHOLE BLOOD SPECIMENS	NO. OF GUINEA	NO CLOT	PARTIAL LYSIS IN 24 HR.			COMPLETE LYSIS IN			
TIME OF COLLECTION	PIGS	LYSIS	+	++	+++	24 HR.	4 HR.	2 HR.	1 HR.
Incubated in Plain Buffer									
Before shock injection	6	5	1						
1 min. after shock injection	7	2		2		2			1
At height of shock	6			1	2	1	2		
At death	7				2	3	1		1
Incubated in Protamine Buffer									
Before shock injection	7	2	2	2	1				
1 min. after shock injection	7	1		1		4			1
At height of shock	7			1	1	1	4		
At death	8				2	3	2		1

DISCUSSION

These results make clear that the abnormal activation of the blood fibrinolysin in samples of clotted guinea pig blood collected during specific anaphylactic shock, previously reported, is in no way peculiar to this reaction, but occurs also in similar degree when normal guinea pigs are subjected to common forms of acute anaphylactoid shock. This is compatible with the view of many investigators that some (at least) of the same basic mechanisms are involved, whether shock is triggered by a specific antigen-antibody complex or by a so-called anaphylactoid agent in the absence of antibody.

Blood specimens collected during trypsin shock showed an extreme degree of rapid spontaneous clot lysis. This is presumably related to the fact that trypsin acts directly as an activator of both human and animal fibrinolysin.[4] It was an early idea that certain similarities between trypsin shock and anaphylactic shock provided a significant clue, indicating the likelihood that a similar trypsin-like proteolytic enzyme may play a key role in the mechanism of anaphylactic reactions.[5] This concept lost much of its cogency, however, when it was shown later that trypsin shock is not caused primarily through histamine release,[6, 7] and thus lacks the most characteristic feature in the pathogenesis of guinea pig anaphylaxis.[8]

More pertinent to the problem of the mechanism of anaphylaxis is the association of fibrinolysis with peptone and anaphylatoxin shock, both of which involve release of toxic quantities of histamine, and closely simulate specific anapylactic reactions. Fibrinolysis after peptone injection was noted early by Nolf[9] and

has since been observed repeatedly in dogs[10, 11, 12] and other animals.[13] Moreover, peptone has been shown to be capable under appropriate conditions of inducing proteolytic activity when added to guinea pig serum[14] or its euglobulin fraction,[15] and fibrinolysis has been regarded as a likely mechanism for the release of histamine from the tissues.[5, 10, 16] Recent studies[17] have supported the conclusion, however, that it is not plasmin (fibrinolysin) that is directly concerned in histamine release, although some kind of enzyme system is almost certainly involved. The effect of peptone on body fluids is complex, causing activation of several enzymes, including esterases derived from precursors different from plasminogen.[15, 18, 19] The damaging effects of intravenous peptone injections cannot therefore be attributed to the activation of fibrinolysin alone, and present knowledge suggests that this enzyme has nothing to do with initiating the shock reaction.[20]

SUMMARY AND CONCLUSIONS

The spontaneous lysis of clotted blood samples collected during acutely fatal shock in guinea pigs following intravenous injection of peptone, histamine, trypsin, and anaphylatoxin was found to be a regular feature of these reactions, just as observed previously in guinea pigs undergoing specific anaphylactic shock. These findings, especially in the case of peptone and anaphylatoxin shock, which so closely simulate anaphylaxis, re-open the question of the significance of fibrinolysis in relation to the fundamental mechanisms of histamine release and the pathogenesis of the ensuing shock. A consideration of the present state of understanding of the blood proteases points to the conclusion that fibrinolysin activation, despite its regular occurrence, is not the primary event in initiating the shock reaction. More study is needed, particularly of the comparative immunological significance of the fibrinolytic enzyme system versus esterolytic enzyme systems.

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EFFECT OF RUBEOLA, RUBELLA, VARICELLA, AND A VIRAL UPPER RESPIRATORY INFECTION UPON THE SEVERITY OF CHILDHOOD ASTHMA: WITH A NOTE ON THE ROLE OF CORTICOSTEROIDS

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IT IS A FAMILIAR clinical paradox that certain infectious processes can either aggravate or ameliorate the syndrome of bronchial asthma.[1] Although such diseases as rubeola, pertussis, and acute respiratory infections have been found to initiate the first asthmatic episode,[2] the reverse has also been reported.[3, 4, 5] The same acute febrile illnesses, as well as local inflammatory disorders, have been noted to produce a temporary interruption in the cycle of recurrent asthmatic attacks, or to reduce their severity. Therapeutic methods like fever therapy and fixation abscess have been based on this observation.[6]

In an effort to divide infections in two groups, depending upon their effect on asthma, Feingold[1] concluded that the childhood exanthemata and possibly other viral illnesses consistently ameliorate asthma, while the respiratory infections associated with leukocytosis, as a rule, aggravate it. There are, however, certain notable exceptions, such as asthma following a presumably viral "common cold," or the absence of wheezing in connection with a bacterial pharyngotonsillitis or pneumonia. In other communications,[3, 7] it was suggested that fever may be the main factor determining whether aggravation or amelioration of asthma will occur during infection.

The number of asthmatic patients now receiving corticosteroids is considerable, probably greater than generally realized. During the years 1958 and 1959, up to 97 per cent of all children admitted to the Jewish National Home for Asthmatic Children, in Denver, gave a history of previous anti-inflammatory steroid therapy. Of them, about 60 per cent were on maintenance steroids at the time of admission.[8, 9] After admission to J. N. H. A. C., a slow withdrawal of the steroid was possible in the majority of cases. Nevertheless, of the 150 children under treatment at the Home, an average of 20 per cent required maintenance doses of anti-inflammatory steroids (averaging 5 to 10 mg. of prednisone or the equivalent per day) for a long period as the most effective means of permitting socially adequate function and relative freedom from acute respiratory discomfort.

The primary interest of this study was to ascertain the effect of certain infectious diseases on asthma and the influence of steroid therapy upon this

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effect. In addition, however, certain observations were made of the effect of anti-inflammatory steroid therapy upon the course and prognosis of the infectious disease itself.

In recent communications, the observation was reported that varicella carries a grave prognosis[10, 11, 12] or can be re-activated[13] in patients receiving corticosteroids. Haggerty and Eley[11] wrote to sixty-five physicians in the United States, Canada, and England, and gathered data on ten fatal cases of varicella among children on corticosteroid therapy, to which they added the two fatal cases that they had observed. In their communication, however, they stressed the fact that fatalities comprised but a small group of the total number of children who developed varicella while on steroid therapy. Robinson and Bukantz[14] sent a questionnaire to leading pediatricians in the United States and Canada to inquire what their policy is when a child on steroid therapy is exposed to or becomes ill with varicella. Of the thirty-five replies received, twelve physicians recommended prompt reduction or discontinuance of steroids, nineteen maintained the drug at previous levels (of these nineteen, eight would be inclined to increase and six to decrease steroids under specific circumstances) and one unconditionally increased steroids during varicella. In three replies, no opinion was expressed. Significantly, sixteen of the thirty-five respondents stressed the fact that their personal experience with this problem was limited, and they expressed their opinion with hesitancy on the basis of the presently available information in the current literature.

It was, therefore, with great interest and care, as well as with some apprehension, that we observed our patients during epidemics of the three exanthematous diseases, rubeola, rubella, and varicella. In addition, certain observations made during an epidemic of an acute undifferentiated upper respiratory infection affecting the majority of the children in the institution were considered pertinent and are included in this communication.

Table I shows the chronological order of these epidemics. In all four instances, there existed an epidemic state of fairly wide proportions throughout the area of Denver and vicinity. All the children at the J. N. H. A. C. attend public school and are naturally exposed to any contagious disease in the community.

TABLE I. EPIDEMICS AT THE JEWISH NATIONAL HOME FOR ASTHMATIC CHILDREN IN DENVER

	TOTAL NUMBER OF CASES	NUMBER OF PATIENTS ON AI STEROIDS*	DURATION
Rubeola	15	3	5/24/58 - 6/30/58
Viral upper respiratory infection	99	26	2/9/59 - 3/6/59
Rubella	37	12	3/7/59 - 5/20/59
Varicella	17	4	6/10/59 - 8/12/59

*Anti-inflammatory steroid.

PRESENT STUDY

In studying the relationship of asthma to the exanthematous diseases, the children in the institution were divided into two groups (Table II).

The first group consisted of children who were not on steroid therapy at the time they became ill with one of the above diseases. A few of them had never received AI steroids,* while most of this group had received the steroids sometime in the past. The steroids had been discontinued, in general, several months prior to the illness, but in one case as recently as one week preceding the illness.

Rubeola.—As indicated in part I of Table II, there were twelve patients with rubeola not on steroids, seven of whom had some asthma during the four weeks preceding the illness, while five had no asthma before the illness. Of these five children who had no asthma, none had asthma during recovery and one of the five experienced some asthma after recovery.

Of the seven children who did have asthma preceding the illness, again, none had asthma during the rubeola and three remained free of asthma for at least four weeks after it, while four developed some asthma after recovery. The most significant observation was that none of the twelve patients experienced asthma during the acute illness.

Part II of Table II and Fig. 4 reveals that the three children on anti-inflammatory steroids had asthma within four weeks preceding the exanthematous illness. This reflects the policy of the institution to employ the anti-inflammatory steroids only when necessary to control severe asthma, while no attempt is made to suppress asthma completely by steroids. Of the three children who developed rubeola while on steroids (prednisone, 5-15 mg. as shown in Fig. 4), all lost their asthma during the disease. One suffered a recurrence of asthma three weeks later, while the other two remained free for a longer period of time.

Rubella.—Of the twelve children who had no asthma previously (part I, Table II), all remained free of asthma during the disease and only two showed some wheezing subsequently. Of thirteen children with asthma prior to rubella, three continued to have asthma during the disease and seven of the remaining ten developed some wheezing later.

Of the twelve on corticosteroids, again all had been asthmatic prior to developing rubella, five of the twelve continued to experience asthma during the illness, and of the seven whose asthma subsided during illness, five again experienced asthma following recovery.

Varicella.— Of the eight children who had not had asthma immediately before varicella, none developed asthma during the disease. Of the five who had experienced asthma before, one maintained some asthma during the disease and one developed some asthma following it.

Of the four children who suffered varicella while receiving steroid therapy (prednisone 5 to 10 mg. daily, as shown in Fig. 5), three continued to wheeze during varicella, and the one who showed no asthma during the disease was asymptomatic when discharged from the institution two weeks later.

The relationship of asthma to the degree of febrile response during the three epidemics is shown in Fig. 1. No patient had asthma during rubeola.

*Anti-inflammatory steroids.

TABLE II. EFFECT OF EXANTHEMATOUS DISEASES ON ASTHMA

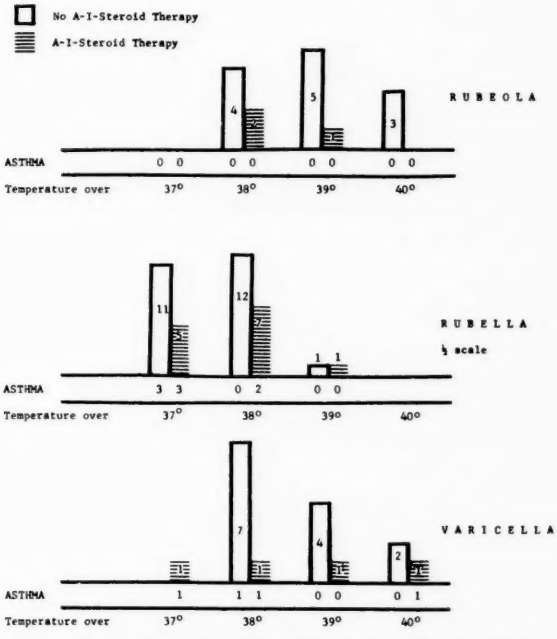
ASTHMA BEFORE (4 WK.)					NO ASTHMA BEFORE (4 WK.)			
NO ASTHMA DURING OR AFTER	NO ASTHMA DURING BUT SOME AFTER	ASTHMA DURING	TOTAL		TOTAL	NO ASTHMA DURING OR AFTER	NO ASTHMA DURING BUT SOME AFTER	ASTHMA DURING
I. Children not on AI Steroid Therapy								
3	4	0	7	Rubeola	5	4	1	0
3	7	3	13	Rubella	12	10	2	0
3	1	1	5	Varicella	8	8	0	0
II. Children on AI Steroid Therapy								
2	1	0	3	Rubeola	0	0	0	0
2	5	5	12	Rubella	0	0	0	0
1*	0	3	4	Varicella	0	0	0	0

*Discharged.

Interestingly some exacerbation was noted the first day during the pre-eruptive stage but always disappeared the next day as the disease progressed. In this respect, our observations confirm the clinical pattern described by Feingold.[1]

Rubella is a milder disease and this is reflected in the lesser temperature elevations seen on the graph. Six children out of sixteen with temperatures below 38° C. had asthma while of the twenty-one with temperature elevation of 38° or 39° C. only two had asthma.

Only one child with varicella in the nonsteroid group suffered from asthma during the disease. Three out of four steroid-treated patients had asthma. Fever in this small sample did not seem to influence the occurrence of asthma.



RELATIONSHIP OF MAXIMUM FEBRILE RESPONSE, ASTHMA AND STEROID THERAPY DURING THREE EXANTHEMATOUS DISEASES.

Fig. 1.—Relationship of maximum febrile response, asthma, and steroid therapy during three exanthematous diseases.

The small number of children here does not permit statistical evaluation of the influence of steroids on the febrile responses. However, observations during an undifferentiated acute respiratory illness which occurred in February, 1959, demonstrated that steroid did not suppress fever. This disease was manifested by fever, general malaise, nasopharyngeal hyperemia, and, in some cases, conjunctivitis. Bacteriologic studies of the nose and throat were negative for bacterial pathogens and leukocytosis was absent. The disease lasted three or four days and recovery was prompt and uneventful. Of the ninety-nine children with this disease, twenty-six were on corticosteroids and seventy-three were not on steroids. As shown in Fig. 2, the maximum febrile responses of the two groups were identical. Steroid therapy, ranging from 2.5 to 15 mg. daily of prednisone, did not seem to influence the maximum febrile response (Fig. 2. *B*).

It was noted that during this illness, children remained exceptionally free from asthma. To verify this observation, the number of bronchodilator aerosols that this group of children required before, during, and after the epidemic were counted (Fig. 3). By this rather crude measurement it was shown that during the peak of the epidemic the need for aerosol therapy dropped precipitously and returned to previous or even higher levels one week later.

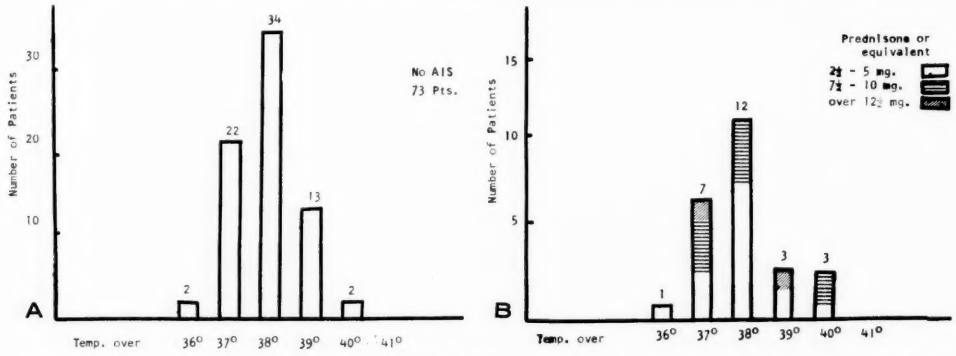


Fig. 2.—*A*, Maximum febrile response of 73 asthmatic children who did not receive AIS therapy during an acute viral epidemic, February, 1959.

B, Maximum febrile response of 26 asthmatic children on long-term AIS therapy during acute viral epidemic, February, 1959.

No.nebs./day
40
30
20
10
Febrile URI Cases
60
40
20
No.of children with fever and characteristic UR symptoms.
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 20 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7
February
March

Fig. 3.—Number of visits to the dispensary for isoproterenol nebulization per day for the entire population (140 children) of the J. N. H. A. C. during the month of February and first week in March, 1959. The incidental course of a febrile upper respiratory epidemic is shown. *URI*—Upper respiratory infection.

Fig. 4 graphically illustrates the clinical course of the three patients with rubeola that were on anti-inflammatory steroid therapy at the time of this disease. These are presented in some detail to illustrate the striking amelioration in the state of asthma,* associated with this disease. The middle portion of the graphs

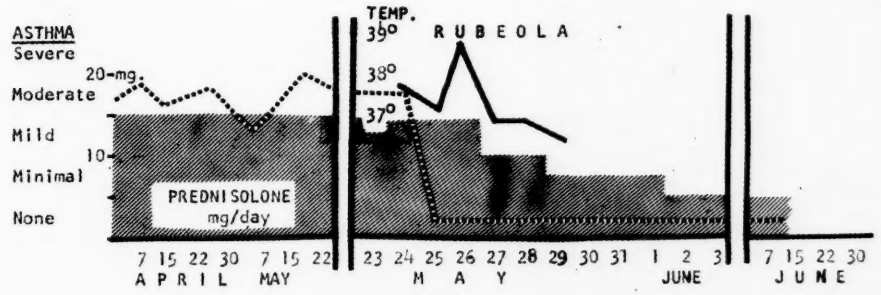
*Asthma was classified as follows: minimal—occasional wheezes audible on auscultation without subjective discomfort; mild—some wheezing, with occasional subjective discomfort (dyspnea, cough, tightness) relieved by inhalation of a bronchodilator, no more than once or twice daily; moderate—scattered numerous wheezes and râles with considerable subjective distress, only temporarily (for a few hours) relieved by injectable bronchodilators; severe—continuous dyspnea and wheezing, not relieved by inhaled or injectable bronchodilators.

has been extended to show the daily data during rubeola, while the patients' previous and subsequent course is condensed and represented as weekly averages.

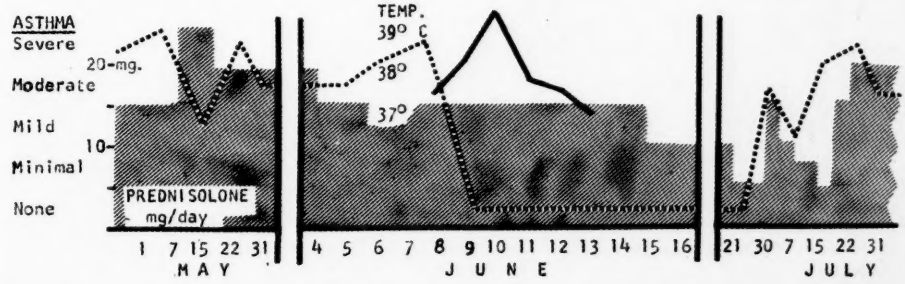
Cases of varicella observed in children on prolonged anti-inflammatory steroid therapy are illustrated in Fig. 5. These are presented to illustrate the uneventful course of the disease and the absence of complications.

CASE REPORTS

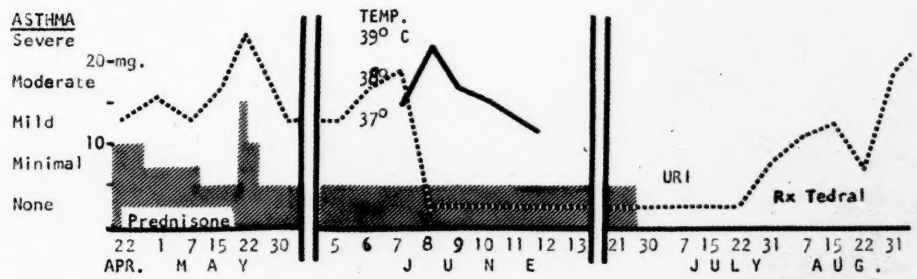
CASE 4.—G. H., a 9-year-old boy, was admitted to the Home on Nov. 13, 1958, with a history of eczema since the age of twelve months and asthma since twenty-four months of



Case # 1.Eight year old girl,asthmatic since the age of 4 years.On intermittent steroid therapy for over 2 years and continuous daily prednisolone for 13 months.

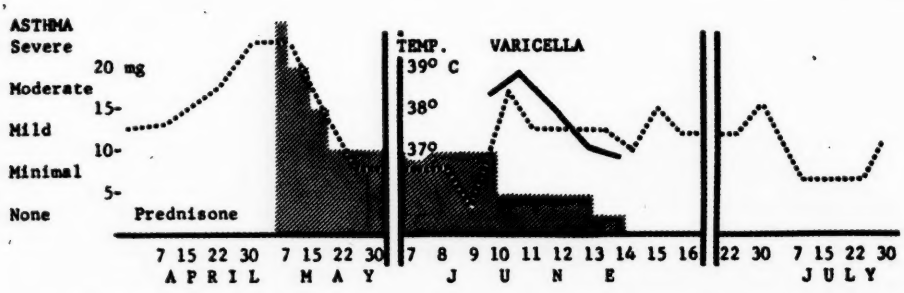


Case # 2.Seven year old boy,asthmatic since the age of 18 months.On continuous anti-inflammatory steroid therapy since the age of 2 years.

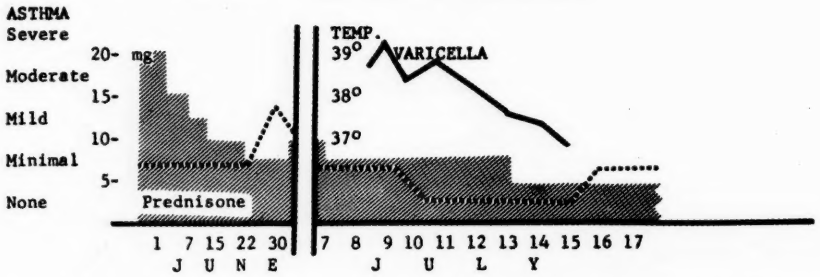


Case # 3.Ten year old girl,asthmatic since the age of 3 years.On continuous anti-inflammatory steroid therapy for 5 years prior to this illness.

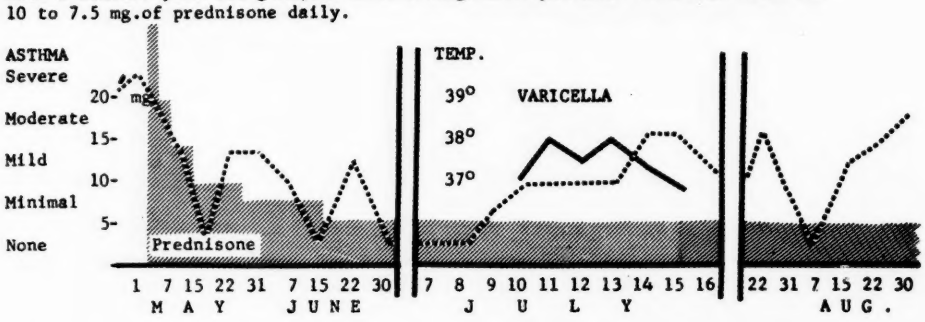
Fig. 4.—Diagrams of three cases of rubeola in asthmatic children on prolonged anti-inflammatory steroid therapy.



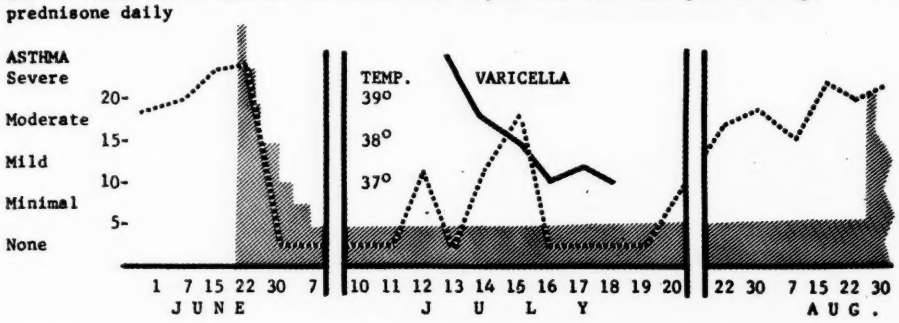
Case # 4:G.M.9 year old boy,who was on prednisone 5 mg.daily at the onset of varicella. The steroid was gradually discontinued.No complications.



Case # 5:K.H.7 year old girl,who went through uncomplicated varicella while on 10 to 7.5 mg.of prednisone daily.



Case # 6:J.S.C.Mild case of varicella in an 8 year old asthmatic girl on 5 mg. prednisone daily



Case # 7:J.A.7 year old boy,who had a moderately severe case of varicella,without complications,while taking prednisone 5 mg.daily.

Fig. 5.—Diagrams illustrating four cases of varicella among asthmatic children on prolonged anti-inflammatory steroid therapy.

age. The asthma had been perennial, worse during August and September for the past several years. The severity of asthma increased at the age of three years and it was at that time that steroid therapy was initiated. On admission to the J. N. H. A. C. he was on prednisolone 10 mg. daily, which he had been taking practically without interruption for more than a year preceding his admission. Prednisolone was gradually reduced and then discontinued on Jan. 2, 1959. He suffered a recurrence of asthma in February, 1959. This responded, initially, to bronchodilator drugs. The frequency and severity of the attacks, however, increased, and on May 5, 1959, anti-inflammatory steroid therapy was re-introduced in the form of prednisone. Initally, he was given 30 mg. for the first twenty-four hours, and then the dose was rapidly reduced to the maintenance level of 10 mg. daily. On that dosage, the asthma was minimal to mild. While still on this dosage of prednisone, he developed, on June 10, 1959, the symptoms and signs characteristic of varicella. As is shown on Fig. 5, prednisone was reduced to 5 mg. daily on June 11, to 2.5 mg. daily on June 13, and was discontinued the following day. Beta hemolytic streptococcus group A and staphylococcus, mannitol and coagulase positive, were isolated from the extensive eczematous skin lesions. He received appropriate local therapy and, in addition, oral penicillin and erythromycin, which produced a rapid clearing of the secondary bacterial infection of his skin. Mild wheezing was present throughout his illness with varicella. Asthma remained mild through the summer of 1959, and an exacerbation was again noted in September and October, 1959. He continued to respond, however, to bronchodilators and no re-introduction of corticosteroid therapy became necessary.

Case 5.—K. H., a 7-year-old girl, was admitted to the J. N. H. A. C. on July 18, 1957, with a history of asthma since the age of ten months. She had not responded to the various anti-asthmatic medications and hyposensitization therapy and had required numerous hospitalizations and frequent courses with cortisone or its analogues, in relatively large amounts (20 to 25 mg. daily). At the time of her admission to the J. N. H. A. C., she had been taking 7.5 mg. of prednisone daily. This was maintained and reduced to 5 mg. daily one week later. As she remained virtually asymptomatic, further reduction was possible to 2.5 mg. daily, on which she was maintained for two months until Oct. 22, 1957. Upon discontinuance of the steroid, however, a marked exacerbation of symptoms was noted and she required another course of prednisone, which was given for four weeks. A similar pattern continued. Prednisone at a dosage of 5 to 10 mg. per day relieved the asthma, but any attempt to discontinue the drug was followed by marked exacerbation of symptoms, which necessitated frequent hospitalizations. The longest she had been off steroids was seven weeks, in April and May, 1958.

On March 17, 1959, while on 10 mg. of prednisolone daily, she developed the first signs of rubella and was admitted to the hospital. She had had some asthma the preceding day, but, since her hospitalization, she remained entirely free of asthma and was discharged three days later, after a very mild course.

On July 9, 1959, while again on 10 mg. of prednisolone daily, she was admitted to the hospital with varicella. Her clinical course is illustrated on Fig. 5. The varicella was noted to be of moderate severity and she showed no evidence of asthma. She remained in the hospital for five days and then returned to her cottage. She was discharged from the institution to her parents on July 17, 1959.

Case 6.—J. S. C., an 8-year-old girl, was admitted to the J. N. H. A. C. on Jan. 26, 1959, with a history of perennial asthma, since the age of eighteen months. She had not responded to any of the conventional methods of treatment, and during the two years preceding admission, 1957-1958, she had been on anti-inflammatory steroids with only few intermissions, with a dosage ranging from 5 to 20 mg. of prednisone daily. Prednisone therapy was continued after her admission to the J. N. H. A. C. Attempts to reduce the dose below 5 mg. daily were followed by marked exacerbation of symptoms. Nevertheless, an attempt was made to reduce and discontinue prednisone, while substituting bronchodilating agents.

Prednisone was discontinued on April 7, 1959. Two days later she was admitted to the hospital with rubella. This disease was mild but, while in the hospital, the patient showed a recurrence of fever with radiologic evidence of right middle lobe pneumonia. The white blood cell count was 14,000 with 83 per cent neutrophils, and cultures from the nose and throat revealed the presence of staphylococcus coagulase and mannitol positive. She was treated with tetracycline by mouth and frequent isoproterenol aerosols and discharged on April 25, 1959. A marked recurrence of asthma was noted in late April and, on May 3, 1959, prednisone therapy was re-introduced, with an initial dosage of 30 mg. which was rapidly reduced to a maintenance level of 5 mg. daily. She was still on 5 mg. of prednisone daily when, on July 10, 1959, she was admitted with a diagnosis of varicella. In addition to the typical signs of varicella, she showed considerable dyspnea and asthmatic wheezes on that day. Her clinical course is illustrated in Fig. 5. Moderate asthma persisted throughout her illness and she was discharged on July 16, 1959, with only a few dry vesicles on her skin, but with still some wheezing present in the chest. The asthma remained essentially unchanged for the following several months.

CASE 7.—J. A., a 7-year-old boy, was admitted to the J. N. H. A. C. on April 3, 1958, with a history of asthma since the age of three years. The attacks of asthma had been mostly nocturnal, and always sudden in onset and termination. A moderate exacerbation of symptoms had been observed during the summer months. The history indicated that a short course of prednisone early in 1957 did not remarkably affect the severity of the attacks.

While in Denver, a marked exacerbation of symptoms was noted in August, 1958, and he was then given a course of prednisone, which was maintained through late fall of that year. He was considerably better during the winter and spring of the following year, 1959, but again, in June 1959, he became severely incapacitated by the frequent and severe attacks of asthma, and on June 20, 1959, prednisone was re-introduced with an initial dose of 30 mg. daily, rapidly reduced to a maintenance level of 5 mg. daily. He was on 5 mg. of prednisone daily when, on July 13, 1959, he developed varicella. The clinical course is illustrated on Fig. 5. For one week preceding the onset of varicella, he had had frequent daily attacks of asthma and isoproterenol aerosols produced moderate relief. He had mild asthma the second and third day of his illness, with varicella, and then remained free of asthma until the day after his discharge from the hospital, when asthma recurred with the usual frequency and severity. He was maintained on 5 mg. of prednisone daily throughout his illness and no complications were noted. The subsequent course was essentially similar to that preceding varicella. The exacerbation of symptoms in August was attributed to ragweed sensitivity for which he was receiving hyposensitization injection therapy.

COMMENT

The role of infectious diseases in the genesis and progression of the syndrome of bronchial asthma, although uncontestably significant,[1, 2, 4, 5, 6] has never been adequately clarified. The interesting observation that certain acute infectious processes can suppress allergic responses was recorded by Von Pirquet (quoted by Urbach[15]) as a result of his observation that a temporary abolition of a positive tuberculin skin reaction followed measles. According to Von Pirquet, this transient "anergy" lasted for about nine days. A similar temporary suppression or amelioration of bronchial asthma by acute febrile illnesses has repeatedly been recorded. The present study offers evidence confirming this clinical impression, and suggests that the improvement noted may be nonspecific, possibly related to the degree of pyrexia or stress. The fact that asthmatic children on prolonged therapy with anti-inflammatory steroids experience the same temporary benefits from such intercurrent illnesses

as those not receiving steroids was of particular interest. The amelioration of asthma during or after a febrile illness or also in relation to other forms of stress (such as, accidental or surgical trauma or an intense emotional experience), has been attributed to a transadrenal effect with activation of the pituitary-adrenal-cortical system.[16] The duration of the remission, often lasting for four to eight weeks, suggests that a "corticoid conditioning" effect, as described by Selye,[16] may be more likely. According to Selye, stress may alter the response of the end organs to the available amount of corticoid hormones, whether endogenous or exogenous, thereby potentiating the anti-allergic or anti-inflammatory effects of these hormones. This may well explain the fact that children who had received cortisone or its analogues continuously, for several years, were able to remain asthma free during the exanthematous disease on dosages of steroids which, when tried repeatedly before, had failed to control the asthma. This concept of conditioning, although it offers a good theoretical model does not explain the changes noted in biochemical terms. It is hoped that future study of factors related to the production, conjugation and utilization, degradation and excretion of corticosteroids, mainly the 17 hydroxycorticoids, will help in understanding this phenomenon and possibly offer additional knowledge about the disease itself.

The possible role of immunologic mechanisms in this respect is also not clear. It is difficult to see how alterations in immune reactions alone could be operative, especially in view of the fact that events entirely unrelated to immunology, like trauma, may produce similar results. Perhaps observations of the responses of asthmatic children to immunization with attenuated live virus vaccines (such as, measles) will offer useful data.

It is known that the central nervous system plays a role in the expression of various allergic disorders and, in turn, that stress affects central nervous system function. Hypothalamic changes have been observed in experimental animals exposed to various forms of stress.[17] Destruction of selected areas in the mid-brain[18] or the hypothalamus[19] was reported to abolish anaphylactic reactions. The possibility must be considered that fever, or stress, may act on the central nervous system either directly or indirectly and thus modify the clinical manifestations of bronchial asthma.

Psychiatric considerations also come into play. A "syndrome shift" has been described[20] between a number of psychiatric and somatic, or psychosomatic, conditions. Such a relationship between asthma and febrile illness, or hospitalization in general, remains to be analyzed.

The second, but by no means secondary, purpose of this communication is to demonstrate that children on prolonged anti-inflammatory steroid therapy, in amounts occasionally required for the control of bronchial asthma (5 to 15 mg. of prednisone or the equivalent) may experience a mild and uneventful course of an exanthematous disease without complications.

The fatalities reported among children on corticosteroids, who become ill with varicella, have been ascribed to a possible suppression of antibody formation,[11] for which the steroid was considered responsible. Although such an

effect of high amounts of corticosteroids has been demonstrated experimentally, it appears doubtful that the clinical use of steroids is associated with suppression of antibody formation. The original disease for which steroids were given, or other forms of therapy, such as radiation,[10] may have been playing a role in the fatal outcome of the cases reported. It is also possible that a hasty reduction or withdrawal of the steroid may have left the organism of the child with varicella more vulnerable to the stress of the disease and without the optimum adaptive support of the adrenal cortical hormones.

SUMMARY

From Jan. 1, 1958, to June 30, 1959, observations were made of the effects of epidemics of rubeola, rubella, varicella, and an acute undifferentiated upper respiratory infection on patients at the Jewish National Home for Asthmatic Children. These observations suggest that:

1. An amelioration of asthma occurs during a number of acute febrile illnesses.

2. Such improvement may be related to the degree of pyrexia and may last from one to six weeks or more.

3. Children on prolonged anti-inflammatory steroid therapy show the same beneficial response to these intercurrent illnesses as do children who have not received steroids.

4. Anti-inflammatory steroids do not necessarily alter the course or mask the symptoms of an acute infection.

5. Prolonged anti-inflammatory steroid therapy, at least in the dosage occasionally required in the management of asthma, does not seem, in the authors' experience, to be associated with an increased incidence of complications of the childhood exanthemata.

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THE RELATIONSHIP OF ALLERGY TO THE COLLAGEN DISEASES

William B. Sherman, M.D., New York, N. Y.

THE CONCEPT of collagen diseases is historically so related to the hypothesis that they are manifestations of hypersensitivity, that use of the term suggests allergy as the cause. Klinge[1] in 1933 pointed out that a number of diseases of unknown etiology, including rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, and periarteritis nodosa showed similar lesions of the collagen tissues, including the walls of blood vessels, generally described as fibrinoid degeneration. At the same time he noted that lesions of this type could be produced experimentally in animals by sensitization to protein antigens and suggested that these human diseases were manifestations of hypersensitivity. Other diseases showing similar lesions of the collagen and included in the concept, either then or later, were dermatomyositis, scleroderma, thrombo-angiitis obliterans, and malignant nephrosclerosis. Since the etiology of all these diseases had long presented unsolved problems, this new hypothesis attracted wide attention among pathologists.

At that time, the pathologic lesions of human serum sickness were not known. In 1937, Clark and Kaplan[2] and, also, Eason and Carpenter[3] reported similar collagen lesions in the arteries of patients dying during the course of serum sickness; the patient of Eason and Carpenter also having rheumatic fever. Both groups of authors noted the resemblance of the arterial changes to Klinge's lesions, but hesitated to describe them as characteristic of serum sickness in man.

It remained for Rich and his associates[4, 5] to definitely establish the relationship of arterial lesions in human autopsy material to serum and drug hypersensitivity reactions, and to reproduce them experimentally in rabbits injected with heterologous serum. They had no hesitation in identifying the experimentally produced lesions in rabbits with those of periarteritis nodosa in man, and, also, described lesions resembling those of rheumatic fever, rheumatoid arthritis, lupus erythematosus, and glomerulonephrits.[6] It was suggested that periarteritis nodosa and possibly other diseases of the group might be cured by application of suitable methods of allergic diagnosis.

The fact that these experimental vascular lesions were indeed due to antigen-antibody reaction was further established by the studies of Dixon,[7] Hawn and Janeway,[8] and others, in which it was shown that such lesions

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were developed at the time of antibody formation, and did not appear after the injection of antigen if antibody production was inhibited by x-radiation or treatment with nitrogen mustards.

While this concept was originated and developed primarily by the pathologists, supporting clinical evidence was offered by Cohen, Kline, and Young,[9] Rackemann and Greene,[10] and Wilson and Alexander,[11] all of whom reported cases of periarteritis developing in patients with pre-existing bronchial asthma. Reimann[12] reported periarteritis complicating trichinosis, a parasitic disease in which there are obvious allergic manifestations.

On the basis of these reports, pathologists, who had long hesitated to diagnose allergy on the basis of anatomic findings, began to interpret collagen degeneration, particularly of the arteries, as evidence of allergic disease. But, in the midst of the wide acceptance of the concepts of Klinge and Rich, a few careful workers in the field have expressed grave doubts. It has been pointed out that the arteritis, or periarteritis, of sensitized rabbits are not *nodosa*, as the small aneurysms noted by Kussmaul and Maier[13] in the spontaneous human disease are lacking.[14] When Rackemann and Greene reported their cases of periarteritis associated with asthma, Libman[15] noted that the arteritis not only lacked the feature of aneurysms, but was essentially a microscopic diagnosis, while the classic type of Kussmaul was readily apparent to the naked eye. Pathologists were also unable to agree as to whether the myocardial lesions described by Rich in rabbits with serum sickness were actually the Aschoff bodies that had long been considered pathognomic of rheumatic fever.

Gitlin and Janeway have said, "Whether or not the so-called collagen diseases are indeed manifestations of a hypersensitivity reaction is not known. It should be recognized with caution that while hypersensitivity can produce lesions in animals that resemble the lesions seen in certain collagen diseases in man, there is actually little or no unequivocal evidence that hypersensitivity is the etiology in humans."[16]

The practicing allergists have devoted considerable attention in recent years to this group of collagen diseases as evidenced by the contents of allergy journals and the programs of allergy societies. Yet it must be admitted that the principles and techniques of clinical allergy have contributed little to the practical diagnosis and treatment of these diseases, as they occur spontaneously in human beings. Only recently, other immunologic techniques are exposing new and exciting aspects of some of the typical collagen diseases, and it seems timely to review realistically the role of allergy in their etiology.

Collagen has been defined as a substance forming the fibers of connective tissue which swells on treatment with dilute acetic acid and which hydrolyzes to gelatin on boiling.[17] To the average physician it is familiar as closely packed fibers, which stain pink with eosin, lying in an amorphous ground substance. With the light microscope, little can be seen of the structure of the individual fibers, but under the electron microscope the fibers show definite, regularly spaced, transverse striations, occurring chiefly at intervals of 650 Å

units. While the diameters of individual fibers vary, the striations of adjacent fibers generally coincide.[18] These striations are believed to reflect the molecular structure of the fibers. The ground substance appears just as structureless under the electron as the light microscope.

In the lesion of fibrinoid degeneration, the collagen fibers swell, change in staining characteristics, become fragmented, and are replaced by an amorphous material believed to be composed partly of degenerated fibrils, but largely of fibrin and other material precipitated in the ground substance.[19] It may be noted that the proteins of the ground substance of connective tissue are apparently derived from the plasma proteins and remain in dynamic equilibrium with the intravascular proteins, so that abnormal proteins in the circulating plasma may enter the connective tissues.

By fluorescent antibody techniques, the presence of gamma globulin in the lesions is readily demonstrated. Dixon and associates[7] have shown that, in the arterial lesions produced in rabbits by injection of bovine serum albumin, both the antigen and rabbit gamma globulin are stained by appropriate fluorescent antibodies. The presence of the bovine serum albumin appears to be directly related to the development of the lesion, since other heterologous proteins to which the rabbit is not sensitized do not enter the lesion if injected into the rabbit's blood stream during the course of its development. This naturally suggests that an antigen-antibody reaction is the basis of the lesion. However, the rabbit gamma globulin is more widely distributed in the lesion than the antigen, and rabbit serum albumin is also present, suggesting nonspecific transudation of the host's plasma proteins, as well as precipitation of antibody.

Doubt is thrown on the importance of gamma globulin in the development of fibrinoid degeneration by the occurrence of such lesions in cases of congenital agammaglobulinemia. Janeway[16, 20] found six cases of what appeared to be rheumatoid arthritis in a group of sixteen boys with congenital agammaglobulinemia. Without analyzing in detail the clinical diagnosis, it may be noted that biopsies from these boys showed typical fibrinoid degeneration of the collagen around the joints, but that no gamma globulin could be stained in the lesions by the fluorescent antibody technique. In view of the unusual incidence of collagen disease in this group, one might well wonder whether the gamma globulin found in fibrinoid lesions is protective rather than causative.

Basically, there are two questions embracing the cardinal points of Klinge's thesis. First, is the lesion of fibrinoid degeneration of collagen sufficiently distinctive to be justified as the basis for the grouping of diseases which vary greatly in their clinical manifestations, prognosis, and even their most distinctive anatomic lesions? Second, if such a classification is warranted, does this histologic evidence afford adequate proof that these diseases are manifestations of allergy?

Several of the best known collagen diseases, such as lupus erythematosus, periarteritis nodosa, and rheumatoid arthritis, may start with confusingly similar clinical pictures of fever, arthralgia, and alterations of the plasma proteins, manifested by an increased sedimentation rate, reversal of the albumin-globulin ratio, increased C-reactive protein, and a variety of nonspecific or

false-positive serologic reactions. Errors in distinguishing the various conditions are common and the baffled clinician may take refuge in the vague diagnosis of "collagen disease." However, when the disease has run its course and the complete pathologic picture is exposed at autopsy, only a few cases are difficult to classify, the great majority showing such specific lesions as the Aschoff body of rheumatic fever, the wire loop capillaries of lupus erythematosus or the small aneurysms of periarteritis nodosa, which carry more diagnostic significance than the collagen changes common to all.

The arteritis of experimental serum sickness in rabbits may be considered the prototype of collagen disease, and similar changes are a striking feature of the Arthus phenomenon. However, lesions identical with fibrinoid collagen degeneration are seen in other conditions of known and varied causes, unrelated to allergic phenomena. Local changes of this type may been seen in the bases of peptic ulcers, in areas adjacent to pancreatic necrosis and acute bacterial infections, and in tissues exposed to radiation.[21] Widespread lesions of the same type are seen in the arteries of animals rendered hypersensitive by the Goldblatt operation[22] or by the injections of desoxycorticosterone.[23] Similar changes are said to be produced in horses by a specific virus infection of the arteries.[24] These and other examples cast doubt on the advisability of considering the presence of fibrinoid collagen lesions as evidence of an etiologic relationship between otherwise dissimilar diseases, and of considering collagen degeneration as evidence of allergy. Klemperer[21] has written that the concept of collagen disease, . . . "is of heuristic value only and will have served its purpose when the abnormalities of the intercellular substances have been fully appreciated and comprehended." Also "Pathogenetic research cannot be advanced . . . (by) . . . interpreting ill defined histologic structural alterations in terms of poorly understood experimental phenomena."

At present it appears doubtful that the etiology of these diseases will be established by histologic studies. The chances seem greater that it will be found in immunologic studies directed at antigens and antibodies involved in the actual human diseases. While such studies are far from their goal of demonstrating the exact pathogenesis of the diseases, they appear at present to differentiate the various collagen diseases rather than to bring them together.

This is illustrated by consideration of four of the most common and most carefully studied diseases of the group—rheumatic fever, rheumatoid arthritis, lupus erythematosus, and periarteritis nodosa.

Of the four, rheumatic fever, which has received the least attention in the writings of the allergists, is the one disease which fits Von Pirquet's[25] original definition of allergy as an example of altered reactivity to an infectious or antigenic agent as a result of repeated contact. The infective agent is the Group A hemolytic streptococcus.[26] Among previously healthy persons suffering from streptococcal tonsillitis, only about 3 per cent of infected patients develop rheumatic fever.[27] In those who do, the rheumatic symptoms develop about one week after the onset of infection, at the interval typically required for antibody formation. This incubation period is identical with that of primary serum sickness, or penicillin reaction, and indeed the symptoms of fever, arthralgia, and

various skin rashes may be sufficiently similar to cause confusion if the streptococcal pharyngitis has been treated with penicillin. A second streptococcal infection in patients who have had one attack of rheumatic fever may be expected to cause a recurrence in 30 to 50 per cent of cases, showing that sensitization has been acquired in the first attack. If the specific cause is avoided by adequate antibiotic prophylaxis, no recurrence is to be expected.

Regarding the antibody mechanism of this sensitization, no information is available. The Group A streptococcus produces a number of exotoxins, of which the best known is streptolysin O. All of these and the intracellular type specific M-substance may produce antibodies which appear in the blood about one week after the onset of infection. However, antibodies appear equally after uncomplicated streptococcal tonsillitis and in acute rheumatic fever.[27]

Although both joint lesions and subcutaneous nodules of rheumatoid arthritis may resemble those of rheumatic fever and cases are sometimes confused in practice, the etiology and the serologic findings are very different. There is no real evidence that the Group A streptococcus, or any other organism, plays a part in its etiology; yet the sera of a high percentage of patients with rheumatoid arthritis will agglutinate Group A hemolytic streptococci,[28] while sera of patients with rheumatic fever do not. On the other hand, the sera of rheumatoid arthritis patients do not contain the antistreptolysins characteristic of streptococcal infections and acute rheumatic fever. Agglutination of the streptococcus by the sera of patients with rheumatoid arthritis is not the effect of specific antibody and a great variety of different particulate materials, such as sensitized sheep cells,[29] latex particles,[30] and red cells coated with Cohn's Fraction II of human gamma globulin,[31] are likewise agglutinated by such sera. The factor in the serum producing these reactions is a 19S gamma globulin[32] found most often in rheumatoid arthritis, but occasionally in patients with periarteritis nodosa, disseminated lupus erythematosus, and other diseases. The factor is absent in children who have both congenital agammaglobulinemia and rheumatoid arthritis. Under suitable conditions it precipitates with Fraction II of human gamma globulin[33] but it is not species specific and will react also with normal rabbit gamma globulin. Adsorption of active serum with cells coated with Fraction II prevents the agglutination of sensitized sheep cells and hemolytic streptococci.[31] The immunologic classification of this "rheumatoid factor" is not clear at present, but it has more of the features of an autoantibody than of one produced in response to any extrinsic antigen or infective agent. Its relationship to the pathogenesis of the typical joint lesions is still obscure.

In lupus erythematosus the serologic findings are equally remarkable.[34] It is well known that the sera of most patients with lupus erythematosus contain a gamma globulin which causes normal leukocytes to phagocytize the nuclei of their mates and assume the form of typical Hargrave's cells. When active sera are incubated with calf, rabbit, or human leukocytes, the active factor is absorbed from the serum. When the leukocytes used for this absorption were washed free of serum and incubated with normal human leukocytes, their nuclei were phagocytized to form L. E. cells.[35]

Active sera were also shown to fix complement in the presence of nuclei derived from many different organs and species of animals and plants. Most of the sera also fixed complement in the presence of desoxyribonucleic acid (DNA) derived from calf thymus, human leukocytes, salmon sperm, and pneumococcus.[36] Red cells coated with DNA were also agglutinated by L. E. sera.[37]

This active material appears to fulfill all the usual criteria of an antibody, but appears unique in reacting alike with autologous, isologous, and heterologous antigens. Within the body it may be expected to act as an autoantibody, presumably to all nuclei and DNA. The possible effects of such an autoantibody might be so varied and severe as to explain the manifestations of clinical lupus erythematosus.

Discussion of periarteritis from the standpoint of allergy depends largely on consideration of pathologic classification. The most widely accepted classification is that of Pearl Zeek,[11] who divides arteritis into five main groups: (1) periarteritis nodosa, (2) hypersensitivity angiitis, (3) allergic granulomatous arteritis, (4) rheumatic arteritis, and (5) temporal arteritis. It is the first four groups that concern us at present. This classification, while based primarily on morphologic changes, fits well with the clinical findings. It does not lump together the inexorable progress and uniform failure to find a specific antigen in typical periarteritis nodosa, with the transitory vascular changes occurring during fever due to specific drug allergy which disappear so rapidly when the drug is eliminated, that they are now rarely seen at autopsy.

True periarteritis nodosa is a progressive disease of the medium-sized arteries, but not of the veins, usually with aneurysmal dilations visible to the naked eye. The pulmonary arteries are usually spared but similar lesions limited to the pulmonary arteries may be seen in certain cases of pulmonary hypertension. At autopsy, lesions in all stages of activity and healing may usually be found in the same case. Leukocytosis is usual, but eosinophilia rare. In cases which develop without previous medication, no specific allergen has ever been incriminated, and it is doubtful if the typical complete pathologic picture can result from drug or serum allergy. It can be produced experimentally in animals by various modifications of the Goldblatt procedure,[22] or by injecting desoxycorticosterone acetate (DOCA) after unilateral nephrectomy.[23]

Hypersensitivity angiitis is an acute necrotizing inflammation of the smallest arteries and veins, with fibrinoid degeneration and perivascular infiltration. At autopsy, all lesions are usually in the same stage of development and are microscopic rather than gross. This is the type of arteritis which occurs in drug allergy and serum sickness and which heals quickly when the cause is removed. It can also occur in lupus erythematosus and rheumatoid arthritis.

The cases of periarteritis developing in chronic asthma[9, 10, 11] are classed as allergic granulomatous arteritis.[38] Here the lesions resemble typical periarteritis nodosa but tend to involve chiefly the smaller arteries, including the pulmonary arteries. Blood eosinophilia is a striking feature, usually 20 per cent or higher. Granulomatous proliferations of epitheloid and giant cells containing masses of eosinophils are commonly associated with this type of arteritis, as is Loeffler's

pneumonia. Since these cases occur in previously allergic people and there is marked eosinophilia, an allergic cause seems most probable, but attempts to demonstrate a specific allergen have been uniformly fruitless. The asthma which precedes the arteritis is usually of the intrinsic type and the arteritis is equally resistant to the allergic approach. Possibly an autoantigen should be sought.

Rheumatic angiitis typically affects the small arteries of the heart and lungs. It is a part of rheumatic fever and its etiology has been discussed above.

SUMMARY

The collagen diseases appear to be a varied group of abnormal states showing no evidence of a common pathogenesis, although several appear to exhibit immunologic features. Even the various types of polyarteritides appear to involve several different mechanisms. In general, more detailed immunologic investigations of the various collagen diseases tend to bring out differences rather than similarities. The only diseases of the group in which specific causative agents have been found are rheumatic fever and certain cases of hypersensitivity angiitis. Autosensitization appears to be a probable pathogenetic factor in lupus erythematosus, and possibly in rheumatoid arthritis, but the immunologic mechanisms of both are only partially understood. The term "collagen disease" appears to have outlived its usefulness as a pathogenetic classification. The etiology of each disease of the group must be considered separately.

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OBITUARIES

CHARLES FRANKLIN MOHR

Charles Franklin Mohr, born Feb. 9, 1905, Providence, Rhode Island, died Aug. 21, 1960, from the effects of a coronary thrombosis, eight years after he left Baltimore, Maryland, to practice internal medicine and allergy in San Diego.

Dr. Mohr's keen diagnostic ability and his scientific approach to therapy were reflections of his varied and excellent training. His residency program was carried out at the Union Memorial and Johns Hopkins Hospitals, Baltimore, immediately after graduation from George Washington University Medical School in 1931. After the completion of his residency, he moved to Boston to work for one year with both Drs. Paul D. White and Elliott P. Joslyn. Thereafter, he returned to the Johns Hopkins Hospital for a year's Fellowship.

He soon became associated with Dr. Joseph Earl Moore, an international figure in syphilology. He found additional time to work in the Allergy Clinic and on the teaching service of the Osler Medical Wards of the Johns Hopkins Hospital. At the time he moved to San Diego he was an Assistant Professor of Medicine, Johns Hopkins University School of Medicine, and Visiting Physician, Johns Hopkins Hospital.

Dr. Mohr's publications revealed the diversity of his curiosity in medical problems, but the majority of his articles dealt with phenomena of immunology. As a teacher he was effectively simple, sound, and direct; as a practitioner, he was scientific, thorough, and sympathetic.

His accomplishments in medicine led to his elections to the American Clinical and Climatological Association and Fellowships in the American Academy of Allergy and American College of Physicians. He was also a member of the San Diego County Medical Association, the San Diego Academy of Medicine, the California Medical Association, the American Medical Association, and the Staffs of Mercy and Sharp Memorial Hospitals.

Dr. Mohr is survived by his wife, Joan, and two children, a daughter, Mary, and a son, David.

E. L. K.

REDFORD A. WILSON

Redford A. Wilson died in Tucson, Arizona, Feb. 13, 1959, of malignant hypertension at the age of 58. Born in Cadiz, Kentucky, in 1900, he entered Vanderbilt University in 1919 and graduated from Vanderbilt University Medical School in 1926. His internship was served at The Louisville

City Hospital and at the New Haven Hospital, New Haven, Connecticut. A Fellowship was taken at Polyclinic, Memphis, Tennessee, in 1928-1929.

Dr. Wilson came to Tucson in 1929 and joined the staff of The Thomas Davis Clinic where he was active until 1953. Highly respected and well known among the medical profession, he was closely identified with St. Mary's Hospital Staff from 1929 until his retirement in 1953, as well as being a member of the staff of The Tucson Medical Center and of The Pima County Hospital. Memberships were held in The American Medical Association and The Arizona Medical Association. He became a Fellow of The American Academy of Allergy and of The American College of Allergists in 1936. He was also a Fellow of The American College of Physicians, of The American College of Chest Physicians, and The American Trudeau Society.

At an early date his keen and searching mind led him into the practice of allergy, a new field of medicine at that time. He was one of the pioneer doctors practicing in the field of allergy in this area.

Dr. Wilson is survived by his wife, Mildred; two daughters, Mrs. R. L. Hollis, Yorktown Heights, New York, and Mrs. J. C. Turpen, Tucson, Arizona; and his mother, Mrs. J. Scott Wilson, Hopkinsville, Kentucky.

W. B. S.

ANNOUNCEMENTS

Mexican Society of Allergy and Related Sciences

At a meeting in July, 1960, the Mexican Society of Allergy and Related Sciences elected the following officers for a two year period.

President, José Luis Cortés
Vice-President, Dr. Emma R. de Cortés
Secretary for the Exterior, Dr. Carlos Abán B
Secretary for the Interior, Dr. Silvia Radillo A
Treasurer, Dr. Graciela Nájera F

The address of the Society is Apartado Postal 30206, México 7, D.F., México.

Meeting of the European Academy of Allergy

A meeting of the European Academy of Allergy will take place in Berlin on April 28 and 29, 1961. The provisional program is as follows.

Friday, April 28, 1961

Main Subject: Differential Diagnosis of Bronchial Asthma and Destructive Emphysema of the Lung.

Speakers: Prof. Gough, Cardiff
Prof. Orie, Groningen
Dr. Scherrer, Bern

Saturday, April 29, 1960

Main Subject: Neurodermatitis and Contact Eczema.

Speakers: Prof. Stüttgen, Düsseldorf
Dr. Schnyder, Zürich
Dr. Dorn, Berlin.

In the afternoon there will be additional lectures and demonstrations—for instance, a film of experiments on nasal allergy will be shown by Prof. Naumann, Würzburg, and a lecture on uveitis will be given by Dr. Witmer, Bern.

Additional information may be obtained from Dr. H. Michel, II. Med. Klinik und Poliklinik der Freien Universität, Berlin-Charlottenburg 9, Spandauer Damm 130, Germany.